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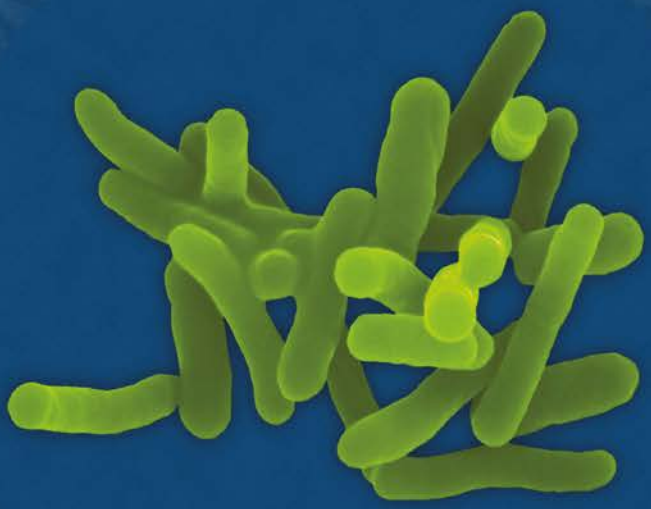
Food and Agriculture Organization of the United Nations

World Health Organization

Microbiological risk assessment of viruses in foods Part 1: Food attribution, analytical methods and indicators

Meeting report



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MICROBIOLOGICAL RISK ASSESSMENT SERIES

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Food and Agriculture Organization of the United Nations
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Declaration of interests

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

Two of the experts declared interest in the topic under consideration. Donald W. Schaffner was involved in consultancy work for the food industry. Sara Monteiro Pires had a close-related involvement in this work. It could not be excluded that the declared interests may be perceived as potential conflicts of interest. In addition, Shannon Majowicz was unable to attend the meeting in the final day due to a prior commitment. Therefore, while they were invited to participate in the meeting, they participated as technical resource people and were 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 present any 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.

Abbreviations

CCFH	Codex Committee on Food Hygiene
CDC	United States Centers for Disease Control and Prevention
DNA	deoxyribonucleic acid
EFSA	European Food Safety Authority
FAO	Food and Agriculture Organization of the United Nations
FIB	fecal indicator bacteria
GC	genome copies
HAV	hepatitis A virus
HBGA	histo-blood group antigen
HEV	hepatitis E virus
HIE	human intestinal enteroid
ISO	International Organization for Standardization
JEMRA	Joint FAO/WHO Expert Meetings on Microbiological Risk Assessment
LOD	limit of detection
LOQ	limit of quantitation
MSC	male specific coliphages
MNV	murine norovirus
ORFs	open reading frames
PMMoV	plant virus pepper mild mottle virus
RNA	ribonucleic acid
RTE	ready-to-eat
RT-PCR	reverse transcription – polymerase chain reaction
RT-qPCR	reverse transcription – quantitative polymerase chain reaction
RT-ddPCR	reverse transcription – droplet digital polymerase chain reaction
TS	technical specification
TuV	tulane virus
U.S. FDA	United States Food and Drug Administration
WHO	World Health Organization

Executive summary

SCOPE AND OBJECTIVES

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 in Rome, Italy from 18 to 22 September 2023, to review recent scientific developments, data and evidence associated with foodborne viruses.

The Expert Committee reviewed the scientific literature on foodborne viruses published since the 2008 JEMRA report on foodborne viruses, and the information submitted in response to a call for data for this meeting. The Expert Committee: 1) reviewed the literature and available surveillance databases, and participated in an expert knowledge elicitation, which ranked foodborne viruses according to frequency and severity; 2) ranked the relevant food commodities of highest public health concern; 3) discussed methods for virus testing performed for outbreak investigation and product testing as part of surveillance and monitoring strategies; and 4) reviewed current and potential indicators for viral contamination.

The Expert Committee decided that water intended for drinking was not within the scope of this Expert 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 AND RECOMMENDATIONS

Foodborne viruses and foods of highest public health concern

The Expert Committee conducted a review of the viruses known to be associated with human foodborne illness. The frequency of illness, and the clinical severity of disease, as well as virus–food commodity pairs, were ranked in the context of foodborne illness. A semi-quantitative approach for an expert knowledge elicitation guided the ranking.

Human norovirus was identified as the leading cause of viral foodborne illness, followed by hepatitis A and hepatitis E viruses. Hepatitis A virus and hepatitis E virus were ranked equally but higher compared to norovirus in terms of clinical severity.

When considering both frequency and severity, the ranking for these viruses fell into three groups as follows:

1. norovirus
2. hepatitis A virus and hepatitis E virus ranked in order
3. rotavirus, sapovirus, enterovirus, astrovirus, and enteric adenovirus ranked in order

The Expert Committee considered commodities from a global perspective and identified the virus–food commodity pairs of highest global public health burden associated with specific viruses (Table 1).

TABLE 1 Virus–food commodity pairs of highest global public health burden

NOROVIRUS	HEPATITIS A VIRUS	HEPATITIS E VIRUS
1. Prepared foods	1. Shellfish*	1. Pork
2. Frozen berries*	1. Frozen berries*	2. Wild game
2. Shellfish*	1. Prepared foods*	

Note: *Substantial regional differences were noted.

The Expert Committee acknowledged the lack of sufficient data to conduct a ranking of foods that may be contaminated by astrovirus, sapovirus, enterovirus, enteric adenovirus, and rotavirus. To address the collective need for more data, countries should enhance investigation of foodborne illness and/or relevant foods for these viruses. Ranking of virus commodity pairs on a global level is challenging; This is partially due to regional differences in foods attributed to human foodborne illness. These differences are in part linked to virus circulation among persons, to regional variations in food consumption and preparation patterns, and to immunity and nutritional status.

Viral foodborne diseases have a substantial impact in terms of morbidity and mortality. Globally, the lack of surveillance data, the potential for asymptomatic shedding, and sparse reporting of foodborne cases pose a major challenge to prevention and control strategies.

Each year, norovirus is estimated to cause 125 million cases of foodborne illness and 35 000 deaths globally. Norovirus is highly infectious, and outbreaks have been linked to foods with low levels of contamination. Viral contamination can occur across the whole food chain. Severe outcomes including hospitalization and death mainly affect children less than 5 years of age, the elderly, and immunosuppressed individuals who may shed the virus for extended periods of time.

Hepatitis A virus is estimated to cause 14 million cases of foodborne illness and 28 000 deaths globally each year and is a reportable disease in some countries. There are significant regional differences in the proportion of hepatitis A cases that are attributed to food due to endemic prevalence and vaccine utilization. International trade of foods plays an important role in transmission to susceptible populations. Wider compliance with international standards, e.g. good agricultural and hygiene practices, is likely to reduce global transmission.

Hepatitis E virus is unique among the foodborne viruses in that it is a zoonotic pathogen with many asymptomatic animal reservoirs, notably swine. While there is no global estimation of cases attributed to food, countries that have investigated further have found that their prior estimates are too low by one order of magnitude or more. Genotypes 3 and 4 originating from infected animals are major agents of foodborne cases of hepatitis E, a trend that has been increasing in recent years in some countries. These genotypes cause acute hepatitis which can be severe in individuals with underlying health conditions. They cause chronic hepatitis leading to cirrhosis and liver damage in people with immunocompromised conditions and are associated with a wide range of neurological sequalae. Undercooked pig products including liver or raw sausage containing liver or blood, as well as liver pâté, are the main foods contaminated by hepatitis E virus.

Analytical methods and indicators for foodborne viruses

Since the 2008 JEMRA report on viruses in foods, international and national standard methods have been developed and validated for detection and quantification of human norovirus and hepatitis A virus in foods. These methods have been implemented in various countries. The International Organization for Standardization (ISO) methods ISO-15216-1:2017 and ISO-15216-2:2019 are widely used for the detection of norovirus and hepatitis A virus in various commodities and are likely to become benchmarks for validation of new methods. Matrices included in these ISO methods are, e.g. leafy greens, soft fruits, and bivalve molluscan shellfish. ISO methods for hepatitis E virus detection in meats and meat products are in development, based on published extraction and RT-qPCR methods with quality controls as defined in ISO-15216-1. National methods, aside from ISO methods, have been validated and are being used by some laboratories. Current standardized methods are based on detection of viral nucleic acid, which does not necessarily indicate infectivity. The methods can be limited by several factors (e.g. the complexity of the food composition, low levels of contamination). Despite the methodological advancements, there remain challenges in their use, most notably ensuring accurate interpretation; application to other viruses and/or matrices; integration of sequencing technologies; and implementation in

low resource countries. Sharing of laboratory and epidemiological data, nationally, regionally, and internationally can improve the understanding and control of foodborne viruses.

A variety of indicators for viral contamination have been investigated, including bacteria, bacteriophages, and plant and animal viruses. Up to this point, these indicators have been mostly studied in environmental waters and bivalve molluscan shellfish, with variable utility. Additional research is needed to determine if there is an appropriate viral indicator for use in other commodities associated with foodborne virus contamination.

Needs assessment and data gaps

There is a need for infectivity assays for wild-type viruses, relative to detection. Despite the existence of multiple experimental approaches, there is still no definitive means to differentiate infectious from non-infectious viruses using molecular amplification. Human norovirus and hepatitis E virus *in vitro* propagation models have been developed but are not yet suitable for routine use.

The Expert Committee recommends that member countries consider capacity-building to support training and adoption of these methods for detecting viruses in foods and the environment. This approach has the potential to enhance knowledge on food attribution, support risk analysis, and reduce the burden of viral foodborne disease worldwide.

Appropriate global actions will help alleviate the anticipated increase in public health risk from viral foodborne illness arising from population growth, the climate crisis, and globalization of food supply chains.

1

Introduction

1.1 BACKGROUND

Problems linked to pathogens in foods, including the associated public health risks and outbreaks of illness, have been reported worldwide. Enteric viruses with foodborne transmission belong to at least ten different families, several of which have been recognized for many years. Virus transmission through foods is complex. Foods can become contaminated at various points along the farm-to-fork continuum, including through interactions with infected individuals at multiple points. Emerging viruses of a zoonotic nature have also been linked to food or postulated to be transmitted via food. Following the COVID-19 pandemic, there is greater public awareness of virus transmission; however, viral causes of foodborne disease are still underreported. Interpretation of global surveillance and reporting systems may be complicated due to limitations in global detection and epidemiological systems. Timely identification of outbreak vehicles is difficult and may be impacted by varying incubation periods.

While there are several viruses that can be transmitted via contaminated foods, human norovirus is considered the leading cause of viral foodborne illness, followed by hepatitis A virus (HAV) and hepatitis E virus (HEV). Hepatitis A virus and hepatitis E virus are both important given the potential clinical severity of illness. Ranking foodborne viruses requires consideration of both frequency and severity. The following viruses are considered in this report: norovirus, HAV, HEV, rotavirus, sapovirus, enterovirus, astrovirus, and enteric adenovirus.

1.2. REQUEST FROM CODEX

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 is to provide direction on how to prevent or minimize the presence of human enteric viruses in foods, most specifically, HAV and norovirus. This guideline is applicable to all foods, with a focus on ready-to-eat food (RTE) 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 norovirus in bivalve molluscan shellfish (Annex I) and an annex on the control of HAV and norovirus in fresh produce (Annex II). These annexes provide additional recommendations for control of these viruses in specific commodities. With the new 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 (FAO and WHO, 2022). The following areas of the guidance were highlighted as needing updating:

- expansion of the scope to address viruses other than HAV and norovirus and emerging vehicles of foodborne illnesses such as frozen fruits;
- revision of interventions in the food chain focusing on process-specific control systems, surface disinfection as well as hand disinfection and food handler hygiene according to available evidence;
- possible inclusion of additional information on testing of foods for enteric viruses with foodborne transmission, considering technical advancements in viral detection in specific commodities and in assessing potential infectivity of viruses; and
- consideration of new scientific findings to control HAV and norovirus in bivalve molluscan shellfish and in fresh produce, made available since the publication of CXG 79-2012, including indicators to monitor seawater quality of mollusc growing areas and risk assessment models.

To support updating the guidelines, the CCFH asked JEMRA to provide scientific advice on items 1, 3 and 4 of the following list:

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.

1.3. DEFINITIONS

Prepared foods is a term used to describe a large, heterogeneous category of foodstuffs that have extensive human handling in preparation that occurs outside of the home, for example, in a restaurant or in a food service establishment.

Ready-to-eat (RTE) foods is a large, heterogeneous category of foodstuffs and can be subdivided in many different ways. According to the Codex definition (FAO and WHO, 2012), RTE foods include any food (including beverages) that is normally consumed in its raw state, or any food handled, processed, mixed, cooked or otherwise prepared into a form in which it is normally consumed without further processing. RTE foods differ in different countries, according to local eating habits, availability and the integrity of the chill chain and regulations specifying, for example, the maximum temperature at retail level.

Water was considered only as that used in food production and processing, as an ingredient, as a vehicle for food contamination, and where water is not the final product that is consumed.

1.4. REFERENCES

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2

Foodborne viruses and foods of highest public health concern

One of the issues raised by the Codex Committee on Food Hygiene at its 53rd session (CCFH53) related to the need for an up-to-date review of the foodborne viruses and relevant food commodities of highest public health concern. To address this issue, the frequency of illness and the clinical severity of disease, as well as virus–food commodity pairs, were ranked in the context of foodborne viral illness. A variety of approaches to attribute foodborne diseases to specific sources are available, including hazard occurrence analysis (the subtyping approach and comparative exposure assessments), epidemiological methods (analysis of data from outbreak investigations and studies of sporadic infections), intervention studies, and expert knowledge elicitations (Pires *et al.*, 2009). Prior to the CCFH53 meeting, the following background documents (Sections 2.1. and 2.2.) were made available and shared with all the experts to facilitate the discussion during the meeting.

2.1. LITERATURE REVIEW FOR OUTBREAK, MONITORING AND SURVEILLANCE DATA

A review of the available scientific literature targeting changes in knowledge since the previous JEMRA meeting (FAO and WHO, 2008) was conducted to develop a bibliography to provide scientific data about outbreaks, monitoring and surveillance. 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 Nations and expert opinions were also relied upon.

The records from Web of Science (n = 6 872) and PubMed (n = 3 007) were added into Distiller. The function "Duplication Detection" was used by comparing the Title, Author and Abstract. A total of 1 261 duplicate articles were found. The "Smart Quarantine" feature in Distiller was used to remove these duplicates resulting in 8 616 publications, which were used to establish the working database for the meeting. The search was carried out on 6 July 2023. 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 781 articles, which 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 was shared before the meeting. The experts had 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.

2.2. DATABASE AND LITERATURE REVIEW FOR GLOBAL BURDEN AND SOURCES OF DISEASE DATA

The preparatory work was done to describe current knowledge on the global and regional burden of disease by viruses commonly transmitted through human sources via the food chain, and on the relative contribution of food and specific foods to this burden. The specific objectives were to:

1. describe available data on the incidence, mortality, and burden of disease caused by foodborne viruses at global and regional levels; and
2. describe available estimates of the proportion of disease by viruses attributable to main transmission routes (i.e. food, environment, direct contact with animals, and human transmission) and to specific foods.

To address objective 1, available estimates on the incidence, mortality, and burden of disease of the list of viruses (norovirus, rotavirus group A, B and C, enteric adenovirus, sapovirus, astrovirus, Aichi virus, HAV, HEV, enterovirus, Nipah virus, highly pathogenic avian influenza virus [HPAI] H5N1, severe acute respiratory disease virus [SARS], coronavirus) from two main sources were reviewed: the Global Burden of Disease Study 2019 (GBD2019) (Abbafati *et al.*, 2019) and the World Health Organization's (WHO's) 2010 Global Burden of Foodborne Disease Estimates (FERG) (WHO, 2015). An additional search was carried out to collect global burden of disease estimates for viruses not included in these two studies.

To address objective 2, a scoping review of source attribution studies published globally between 2000 and 2023 was conducted. A PubMed search was performed by creating exploratory search strings, piloting them, and updating and choosing the search string that yielded the best results. From the studies that fulfilled the inclusion criteria, source attribution proportion estimates and, when relevant, other epidemiological metrics were extracted. The details of this procedure are described in Annex 2.

2.3. EXPERT KNOWLEDGE ELICITATION

During the meeting, a one-day expert knowledge elicitation workshop was held, which applied a structured approach to facilitate the decision-making process.

In this exercise, the answers of the experts were combined to estimate the relative magnitudes of factors through pair-wise comparisons. Each of the respondents compared the relative importance of each pair of items using a specially designed questionnaire.

All the experts were asked to conduct a comparative analysis of the frequency of cases and clinical severity of pairs of foodborne viruses related to foodborne outbreaks, based on their expertise. First, the experts discussed which viruses should be included in the exercise, and eight viruses (norovirus, HAV, HEV virus, rotavirus, sapovirus, enterovirus, astrovirus and enteric adenovirus) were selected from a global perspective. Then, each virus was compared one by one to the other viruses to determine which one causes more foodborne illness cases annually and which one causes more severe outcomes in humans. This process was repeated for every combination of the selected viruses, and the experts considered the outcomes of the exercise and debated them together to reach their decisions. As a result, human norovirus was identified as the leading cause of viral foodborne illness globally, followed by HAV and HEV. Hepatitis A virus (HAV) and hepatitis E virus (HEV) were ranked equally but higher than norovirus in terms of clinical severity. When considering both frequency and severity, the ranking of these viruses fell into these three groups:

1. human norovirus
2. HAV and HEV ranked in order
3. rotavirus, sapovirus, enterovirus, astrovirus, and enteric adenovirus ranked in order

2.4. VIRUS-COMMODITY COMBINATIONS

The Expert Committee considered commodities from a global perspective and identified the virus–commodity pairs of highest global public health burden associated with specific viruses (Table 1).

The Expert Committee acknowledged the lack of sufficient data to conduct a ranking of foods that may be contaminated by astrovirus, enteric adenovirus, enterovirus, rotavirus and sapovirus. It was noted that, to address the collective need for more data, countries should enhance their investigations into foodborne illness and/or relevant foods for these viruses. The ranking of virus commodity pairs on a global scale is challenging, partially due to regional differences in the relative importance of foods associated with illnesses. These differences are in part linked to virus circulation among persons, regional variations in food consumption and preparation patterns, and immune and nutritional status.

2.5. REFERENCES

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3

Human norovirus

3.1. INTRODUCTION

Human norovirus is a major cause of foodborne illness worldwide (WHO, 2023) and is estimated to cause approximately 685 million cases and 212 000 deaths of acute gastroenteritis per year (Kirk *et al.*, 2015). The symptoms of norovirus infections are nausea, vomiting, non-bloody diarrhea, and abdominal pain, which are all self-limiting (WHO, 2023).

Noroviruses are non-enveloped, single-stranded RNA viruses of the family *Caliciviridae* and are classified into ten genogroups (GI-GX) and 49 genotypes (9 GI, 27 GII, 3 GIII, 2 GIV, 2 GV, 2 GVI and one genotype each for GVII, GVIII, GIX [formerly GII.15] and GX) (Chhabra *et al.*, 2019). Genogroups I, II, IV, VIII, and IX infect humans, with GI and GII being the most common. Other genogroups have been found in a broad range of animals including cattle and sheep (GIII), cats and dogs (GIV, GVI, and GVII), rodents (GV), bats (GX), harbour porpoises (genogroup not assigned [GNA1]) and sea lions (GNA2). Human norovirus genotypes have been detected in animal species, although cross-species transmission from animals to humans does not appear to occur (Villabruna, Koopmans and de Graaf, 2019). In humans, GII.4 is the most commonly detected genotype and responsible for the majority of norovirus outbreaks. In human experimental infection studies, the estimated infectious dose ranged between 18–2 800 genome copies of GI.1 or GII.2 (Teunis *et al.*, 2008; Atmar *et al.*, 2014). Infected individuals shed virus both in feces (10^5–10^{11} viral copies per gram) and vomitus (10^3 –10^6 gene copies per mL). Shedding occurs for two to four weeks, with peaks occurring 2–5 days following infection (Melhem, N.M. ed., 2019, Chapter 3).

Transmission occurs through the fecal-oral route via direct person-to-person contact or indirectly by consumption of contaminated food or water or contact with contaminated surfaces (fomites). Foods can become contaminated throughout the entire food chain, during production (e.g. contaminated waters), harvest (e.g. through utensils, contact surfaces, or human hands during harvest and transportation), processing (e.g. from infected food handlers, contaminated processing waters, proximity to vomiting events), or preparation (e.g. infected food handlers) (Bozkurt *et al.*, 2021).

One study investigated the global norovirus genotype profiles associated with foodborne transmission in the period 1999 to 2012 using transmission and genotyping information from three outbreak surveillance systems (NoroNet, CaliciNet, EpiSurv) and from a systematic review of peer-reviewed literature (Verhoef *et al.*, 2015). This study showed that the proportion of outbreaks is dependent on the genogroup and/or genotype (Verhoef *et al.*, 2015). Ten percent of all genotype GII.4 outbreaks, 27 percent of outbreaks caused by all other single genotypes, and 37 percent of outbreaks caused by mixtures of GII.4 and other noroviruses were attributed to foodborne transmission.

3.2. DETECTION IN FOODS

Foodborne norovirus outbreaks have been associated with a broad range of food items, but three categories were recognized as most relevant during the expert knowledge elicitation: prepared ready-to-eat (RTE) foods, frozen berries, and bivalve molluscan shellfish. RTE foods are consumed without a terminal heating step, so if these foods are contaminated during preparation, infectious virus will be consumed (Gagné, Savard and Brassard, 2022).

Food and food products are important transmission vehicles which may become contaminated by contact with contaminated environmental surfaces in food preparation areas. Food handlers pose a major risk in the food preparation chain (Todd *et al.*, 2007); the U.S. FDA Food Code clearly advises people with gastrointestinal infection be subjected to a 48-hour exclusion period from work to prevent potential contamination of food with human norovirus. The Codex Virus guidelines (FAO and WHO, 2012) state that people who have had gastroenteritis should only be allowed to return to work after a period without symptoms of diarrhea and vomiting. Norovirus was detected in feces from food handlers and health care workers linked to gastroenteritis outbreaks in Catalonia from 2010–2012; 59.1 percent were positive for norovirus, more than 70 percent of which were asymptomatic (Sabrià *et al.* 2016). Numerous studies have investigated

the risks attributed to contaminated agricultural water (Sobolik *et al.*, 2021). Norovirus RNA was also detected on the hands of agricultural workers. Therefore, they have also been suggested as a source of contamination for RTE produce (Sobolik *et al.*, 2021).

Many studies on the presence of norovirus in bivalve molluscan shellfish have been reported since the last JEMRA meeting. The presence of norovirus RNA has been detected in shellfish samples around the world, as shown by the following selection of studies where the samples were not implicated in outbreaks. In Spain, norovirus GI was the most prevalent in bivalve molluscan shellfish (32.1 percent), followed by norovirus GII (25.6 percent) (Polo, Varela and Romalde, 2015). Similarly, norovirus was detected in 30 percent (23/77) of shellfish samples in Morocco (Benabbes *et al.*, 2013), 17.3 percent (13/75) of shellfish samples in Thailand (Kittigul *et al.*, 2022). In China, norovirus was detected in 20.7 percent of 480 oyster samples, with no significant differences in prevalence across sampling sites (restaurants, markets and farms) (Tan *et al.*, 2018). In India, 41.3 percent (43/104) of various seafood samples, including shrimps, finfish, clams, and oysters tested positive for norovirus GII (Das *et al.*, 2020).

Norovirus GI and GII RNA have been detected in various fresh produce, including soft fruits, herbs and green leafy vegetables, across multiple countries (see Table 2). In Egypt, fresh strawberry had a norovirus GI prevalence of 25 percent for GI and 40 percent for GII, while herbs (watercress, leek, coriander, and parsley) showed 20 percent for GI and 30 percent for GII(Elmahdy *et al.*, 2022). A study found that 5.3 percent of lettuce samples (30/568) were norovirus positive, mostly from lettuce grown locally, with GI predominating. Norovirus was also detected in 2.3 percent (7/310) of fresh raspberries samples and 3.6 percent (10/274) of frozen raspberries samples (Cook, Williams and D'Agostino, 2019). Similarly, in a fresh produce prevalence study, norovirus was detected in 28.2 to 50 percent of leafy greens tested (867 samples) from Canada, Belgium and France, and in soft red fruits at prevalences of 34.5 percent and 6.7 percent (180 samples) from Belgium and France, respectively. A total of 55.5 percent of other fresh produce types (57 samples; cucumber, tomatoes and fruit salads) analysed from Belgium were found positive (Baert *et al.*, 2011). However, it is important to note that detection of RNA does not necessarily indicate the presence of infectious virus, and outbreaks are not frequently reported for all the fresh produce items that tested positive for norovirus.

3.3. CONTAMINATION LEVELS IN FOODS

The contamination levels of norovirus found in foods can vary (Table 2), as a function of food product and detection techniques used. Notably, the degree of contamination within batches may be non-homogeneous. In bivalve molluscan shellfish, contamination levels vary and depend on the water quality of growing waters. For example, in Brazil, levels ranged from undetectable to 10^7 GC/g digestive tissue (do Nascimento *et al.,* 2022). Other studies reported contamination levels up to 1.5 to 10^4 GC/g digestive tissue in RTE oysters from the United States of America, China and Thailand (Woods *et al.*, 2016; Tan *et al.*, 2018; Kittigul *et al.*, 2022), and levels ranging from 8.7×10^3 to 3.2×10^4 GC/g in sea urchins from Portugal (Santos Ferreira *et al.*, 2020).

In a Dutch study the contamination levels in RTE mussels and oysters varied by sampling location (post-harvest, dispatch centres and retail stores), ranging from 1.69 to 2.52 mean log10 GC/g (Dirks *et al.*, 2021). During a European coordinated monitoring programme on the prevalence of norovirus in oysters, 2 180 samples were taken from production areas and 2 129 from dispatch centres over a two-year period. The prevalence at production areas and dispatch centres were estimated to be 34.5 percent and 10.8 percent respectively. Importantly, the analyses also showed a strong seasonal effect, with higher norovirus contamination levels from winter to spring, with mean contamination levels of 3.37×10^2 GC/g in production area samples and 1.69×10^2 GC/g in dispatch centres (EFSA, 2019).

In berries, virus contamination levels also vary but are usually much lower than in bivalve molluscan shellfish. For example, a study on Canadian cranberries reported 3 to 7 GC/g in the 3 out of 234 samples that tested positive (Chatonnat *et al.*, 2023). A frozen batch of strawberries implicated in a large outbreak in Germany had contamination levels was 2.57×10^2 GC/25 g of GII in positive samples (Bartsch *et al.*, 2018). However, higher contamination levels have also been detected, for example, in strawberries in Egypt which tested positive at 10^4 GC/g but were not associated with a reported outbreak (Elmahdy *et al.*, 2022).

In vegetables and leafy greens in Egypt, norovirus contamination levels ranged from 10^2 to 10^4 GC/g (Elmahdy *et al.*, 2022), while in lettuce in China contamination levels were 9.3×10^3 GC/g for GI and 4.7×10^3 GC/g for GII (Xie *et al.*, 2021).

TABLE 2 A selection of studies of norovirus GI and GII prevalence, detection and contamination levels in different foods

FOOD TYPE	IMPLICATED IN AN OUTBREAK	METHODOLOGY	SAMPLES TESTED POSITIVE	CONTAMINATION LEVELS REPORTED	REFERENCES
Cranberries in Canada		ISO 15216-1, RT-qPCR	GI 3/234 GII 0/234	GI: 3–7 GC/g	Chatonnat *et al.*, 2023
Frozen raspberries in Canada	yes	Magnetic Silica Bead and RT-qPCR	6/8 sample lots of 25 grams were positive for norovirus	GI: Ct 32.9–40.8 GII: Ct 34.4–37.6	Raymond *et al.*, 2022
Fresh strawberries and green leafy vegetables in Egypt		RT-qPCR	Fresh Strawberries: GI 25% GII 40% Green leafy vegetables: GI 20% GII 30%	Fresh strawberries: GI 9.7×10^2 GC/g; GII 2.4×10^3 GC/g. Green leafy vegetables: GI 1.1×10^4 GC/g; GII 2.03×10^3 GC/g	Elmahdy *et al.*, 2022
Oysters in Thailand		RT-qPCR	GII13/75	GII: $8.83–1.85 \times 10^4$ GC/g of digestive tissues	Kittigul *et al.*, 2022
Fresh berries in Singapore		ISO 15216-2 and RT-qPCR	GI: 26/68 GII: 9/68	Most positive samples below the limit of quantification (< 1.2×10^2 GC/g) 1 strawberry sample: 2.5×10^2 GC/g	Eshaghi Gorji *et al.*, 2021
Ice pops in southern Brazil	yes	RT-qPCR	3/7 batches were positive for GII	GII 1.4×10^1–7.6×10^3 GC/ml	Fumian *et al.*, 2021
Frozen strawberries in Germany	yes	RT-dPCR and RT-qPCR	One batch	GII: RT-dPCR; 1.9×10^2 GC/25 g RT-qPCR; 2.6×10^2 GC/25 g	Bartsch *et al.*, 2018
Oysters in China		One-step RT-ddPCR	GI: 2/480 GII: 94/480	1.93×10^4 GC/g of digestive glands	Tan *et al.*, 2018
Dried shredded seaweed in Japan	yes	A3T method, nested RT- PCR	7/21 samples were positive for GII	GII: 3.6×10^2–$2{,}9 \times 10^3$ GC/g	Sakon *et al.*, 2018
Oysters in the United States of America	yes	RT-qPCR		GII: 2.4–82.0 RT-qPCR U/g of digestive diverticula GI: 1.5–29.8 RT-qPCR Units/g of digestive diverticula	Woods *et al.*, 2016

TABLE 2 A selection of studies of norovirus GI and GII prevalence, detection and contamination levels in different foods (cont.)

FOOD MATRIX/ FOOD#	IMPLICATED IN AN OUTBREAK	METHODOLOGY	SAMPLES TESTED POSITIVE	CONTAMINATION LEVELS REPORTED	REFERENCES
Bivalve molluscan shellfish in Spain		ISO/TS 15216-1:2013 RT-qPCR	GI: 54/168 GII: 43/168	10^2 to 10^3 GC/g of digestive tissues	Polo, Varela and Romalde, 2015
Bivalve molluscan shellfish in France		RT-qPCR		1.0×10^2–1.0×10^3 GC/g of digestive tissues	Benabbes *et al.*, 2013
Oysters in Europe		ISO/TS 15216-1:2013 RT-qPCR	Prevalence at production areas was estimated to be 34.5% (CI: 30.1–39.1%), from dispatch centres it was 10.8%	3.37×10^2 GC/g in production area samples and 1.68×10^2 GC/g in batches from dispatch centres	EFSA, 2019

Sources: See References.

3.4. CONTAMINATION LEVELS IN THE ENVIRONMENT AND ENVIRONMENTAL PERSISTENCE (IN REGARD TO POTENTIAL TO CONTAMINATE FOODS)

Human norovirus is known for its high stability in the environment and its notable resistance to inactivation by commonly used disinfectants (Alex-Sanders *et al.*, 2023; Zhai *et al.*, 2023). The high environmental stability of human norovirus is reviewed elsewhere (Cook, Knight and Richards, 2016) but is generally in the range of days to weeks at room temperature. This means that the virus can persist on various contact surfaces for long periods, increasing the likelihood of transfer from the environment to individuals and between fomites and foods, especially in food preparation environments (Derrick *et al.*, 2021).

Norovirus has been found in various water sources (Khamrin *et al.*, 2020), including drinking water (Victoria *et al.*, 2010), estuarine water (Hernandez-Morga *et al.*, 2009), river water (Boonchan *et al.*, 2017), marine and fresh water (Wyn-Jones *et al.*, 2011) and wastewater (Pouillot *et al.*, 2015). A systematic review of human norovirus contamination in water sources was performed by Ekundayo *et al.* (Ekundayo *et al.*, 2021). Notably, human norovirus was included as a reference pathogen for potable water reuse in the WHO guidelines (WHO, 2017; Seis *et al.*, 2022).

The persistence of human norovirus in water is a critical factor contributing to its transmission to raw agricultural commodities (Anderson-Coughlin *et al.*, 2023).

Some studies have demonstrated that norovirus remains detectable by RT-PCR for over 100 days in various water sources, including mineral water, tap water, river water, and treated sewage effluent, at different temperatures (4 °C, 15 °C and −20 °C) (Kauppinen and Miettinen, 2017; Ngazoa, Fliss and Jean, 2008), and even in groundwater, filtered to the United States Environmental Protection Agency (EPA) drinking standards, for more than three years (Seitz *et al.*, 2011). Furthermore, human norovirus shows some resistance to inactivation during wastewater treatment, and a potential for environmental dissemination through natural waters, which could pose significant challenges to controlling contamination of foods, particularly at the pre-harvest phase (Ekundayo *et al.*, 2021).

The presence of human norovirus in river and estuarine waters is primarily attributed to sewage pollution or inadequately treated wastewaters, often leading to contamination of aquatic foods harvested from estuaries and coastal waters that receive wastewaters (Ekundayo *et al.*, 2021). Attention to the quality of water used in aquaculture is also important. For Northern Europe several studies showed that norovirus contamination of shellfish is correlated to the winter season (Lowther *et al.*, 2012). A systematic literature review and meta-analysis showed that sewage samples display higher GII norovirus mean contamination levels during spring (5.3 $\log_{10}$ GC/L) and winter (5.1 $\log_{10}$ GC/L) than in summer (4.3 $\log_{10}$ GC/L) and autumn (4.1 $\log_{10}$ GC/L) (Eftim *et al.*, 2017). However, regional differences in seasonality of documented norovirus outbreaks have been described (van Beek *et al.*, 2018).

Considering environmental contamination, virus particles can be present in high quantities in the feces and vomit of infected individuals and can disperse in the air as aerosols during toilet flushing (Johnson *et al.*, 2013) and aerosolized vomitus (Alsved *et al.*, 2020). Epidemiological evidence suggests that aerosolized vomitus can lead to outbreaks (Godoy *et al.*, 2016) as aerosolized virus particles contaminate environmental surfaces which serve as fomites for virus transfer (Zhai *et al.*, 2023; Derrick *et al.*, 2021; Alex-Sanders *et al.*, 2023).

While implementing strict hand hygiene practices and thorough surface disinfection can diminish virus transmission risk, depending on the type of disinfectant, and policy and/or adherence to best practices, even these may not eliminate virus transmission. Certainly, in enclosed and partially enclosed spaces, especially in high-risk areas (e.g. restrooms) and settings (e.g. health care and food service), precautionary measures are essential and include those such as assurance of adequate hand hygiene, regular cleaning and disinfection of surfaces, ensuring adequate ventilation, and providing health education to workers (Zhai *et al.*, 2023).

3.5. SPECIFIC DETECTION METHODS AND MOLECULAR TYPING

Several validated methods exist for concentrating and extracting norovirus from food specimens, as well as for its detection (see Chapter 8).

Sequencing of clinical – when levels allow – food and environmental samples is used for genotyping. Since the mid-1990s, norovirus genotypes have been defined based on the complete VP1 sequence. However, as there is frequent occurrence of recombination between the open reading frame 1 (ORF1) and ORF2, a dual nomenclature for norovirus classification using both sequences encoding RdRp and sequences encoding VP1 is now being used (ORF1-RdRp=P type, ORF2=genotype) (Chhabra *et al.*, 2019; Kroneman *et al.*, 2013).

Currently, nucleotide sequences of relatively small regions of ORF1 and/or ORF2 of the norovirus genome are often used to genotype strains and identify outbreaks. Typing tools such as the NoroNet (RIVM, 2023) and CDC-typing tool (Tatusov *et al.*, 2021) allow for an international uniform nomenclature, and surveillance networks such as NoroNet and Calicinet allow sharing of genomic data (van Beek *et al.*, 2018; Calderwood *et al.*, 2022).

Whole genome sequencing of norovirus is increasingly being used for human clinical samples which will benefit outbreak tracing and allow for a better understanding of the global epidemiology. However, whole genome sequencing of norovirus from food samples is challenging due to the low virus concentrations, and this holds true for most foodborne viruses.

3.6. HOST SUSCEPTIBILITY AND PATHOGENESIS

Susceptibility to Norovirus

Histo-blood group antigens (HBGAs) are a diverse family of carbohydrates that determine our blood type and can act as host receptors for human norovirus. HBGAs are expressed on mucosal surfaces as well as present in saliva and the gut mucosal linings, and they facilitate norovirus attachment and entry. The expression of HBGAs in humans is governed by genetic variation at the locus coding for these genes and the presence of a FUT2 gene. The FUT2 gene encodes fucosyltransferase 2, which adds fucose to a precursor carbohydrate molecule (Melhem, N.M. ed., 2019, Chapter 2 and 3). This leads to the construction of different ABH HBGAs, which are then expressed on mucosal surfaces. Individuals without a functional FUT2 gene do not express fucosyltransferase2.

These individuals are referred to as "non-secretors" since they do not express most HBGAs on their mucosal surfaces (Faden and Schaefer, 2021). Non-secretors make up approximately 20 percent of the population and appear to be more resistant to infection with certain norovirus strains. For example, non-secretors have been shown to be resistant to infection by norovirus GI.1 (Lindesmith *et al.*, 2003). However, other norovirus strains can infect non-secretors; this is due to their ability to bind to other HBGAs, such as Lewis antigens, that do not require fucosyltransferase for their assembly (Huang *et al.*, 2003; Huang *et al.*, 2005; Lindesmith *et al.*, 2005; de Rougemont *et al.*, 2011).

The genotype and strain-specific HBGA binding specificity combined with the differences in the expression of HBGAs provides a unique, strain-specific pool of susceptible individuals for each virus (Lindesmith *et al.*, 2008; Ruvoën-Clouet, Belliot and Le Pendu, 2013). For example, mutations near the receptor binding domains of GII.4 can either restrict or expand the HBGA repertoire, thus modulating the pool of susceptible hosts (Melhem, N.M., ed. 2019, Chapter 3). This appears to be an important factor in strain evolution.

Clinical manifestations, pathogenesis and shedding

Norovirus infection is typically characterized by a sudden onset of vomiting, watery, non-bloody diarrhea, abdominal cramps, fever and malaise (Hall *et al.*, 2012; Atmar *et al.*, 2006) with an average duration of symptoms of 44 hours (95 percent CI: 38.9-50.7) (Devasia *et al.*, 2015) and an incubation period of 12–72 hours (Lee *et al.*, 2013).

While norovirus illness affects all age groups, the severity of disease differs by age. Comparatively, children suffer high incidence of norovirus gastroenteritis, outpatient and emergency department visits and hospitalizations (Lopman *et al.*, 2011; Gastañaduy *et al.*, 2013; Kotloff *et al.*, 2013; Phillips *et al.*, 2010). Noroviruses typically cause acute, self-limiting infections among immuno-competent individuals; however, elderly patients, immunocompromised and malnourished individuals may experience more severe and prolonged disease, including the potential for chronic diarrhea (Melhem, N.M., ed. 2019, Chapter 4; Lucero *et al.*, 2021). Other complications associated with norovirus infections are necrotizing enterocolitis in preterm infants, and benign infantile convulsions with gastroenteritis (Petrignani *et al.*, 2018).

Data from human intestinal biopsies and the human intestinal enteroid (HIE) culture system (Estes *et al.*, 2019, Ettayebi *et al.*, 2016) showed that human norovirus replicates in intestinal enterocytes; moreover, the virus can be found in the duodenal, jejunal, and ileal segments of the small intestines of infected

individuals (Melhem, N.M., ed. 2019, Chapter 4). Histological changes of the small intestine, such as broadening and blunting of villi, shortening of the microvilli, invasion of inflammatory cells and down regulation of tight junction proteins, which are suggested to increase the permeability of the small intestine, as well as diffusion of water and ions, all seem to contribute to diarrhea (Troeger *et al.*, 2009; Mumphrey *et al.*, 2007). Infection of HIEs can be used to investigate factors driving the infectious period of norovirus gastroenteritis to guide infection control. They may also be helpful in assessing the ability of asymptomatic patients to transmit the virus.

3.7. REGIONAL ISSUES AND DIFFERENCES

In most regions norovirus circulates year-round, although spatio-temporal trends exist. In the Northern Hemisphere, for which the most data are available, norovirus disease burden peaks in the winter months and was positively associated with rain in the wettest month (Ahmed, Lopmand and Levy, 2013). In the United States of America, for example, norovirus seasonality is highest in October to May, and such seasonality is less pronounced in western parts of the United States of America and more pronounced in the north-eastern United States of America (Kambhampati *et al.*, 2023). In Cameroon, norovirus was detected throughout the year but peaked at the beginning of the rainy season (Ayukekbong *et al.*, 2014). However, increases in the number of outbreaks reported can also be observed with the introduction of new strains. Most genotypes and GII.4 variants are detected globally, but also here regional differences exist (de Graaf *et al.*, 2015; CDC, 2013; van Beek *et al.*, 2018; Chan *et al.*, 2017).

Monitoring for norovirus, in humans and in foods (Shah *et al.*, 2017; Jin *et al.*, 2020) usually involves traditional laboratory-based testing (CDC, 2023; Hughes *et al.*, 2021), sometimes supplemented with novel approaches like symptom surveillance or wastewater monitoring (Santiso-Bellón *et al.*, 2020; Edge *et al.*, 2006; Lun *et al.*, 2018; Mabasa *et al.*, 2022). But data on norovirus circulation is lacking in many regions of the world (Kirk *et al.*, 2015), as not all countries have the capacity to implement surveillance and monitoring. For example, in several low- and middle-income regions such as many countries in Africa, there are virtually no data on disease burden as norovirus is rarely diagnosed and formal records or sentinel surveillance programmes are absent. Such a lack of data on human cases is an issue for norovirus vaccine development and deployment (Green, 2018; van Beek *et al.*, 2018; Fisher, Rasmussen and Fonager, 2019). Additionally, without knowing the extent of norovirus in humans and foods, or what burden norovirus poses to the health care and food systems, risk managers, policymakers, and other stakeholders are not

able to adequately prioritize norovirus prevention and control measures (Harris, 2016; Hakim *et al.*, 2018).

Norovirus contamination of the food supply chain poses risks in all regions of the world. As described above, food monitoring/inspection, screening of food handlers, and human health surveillance vary regionally in both organization and capacity. Furthermore, the variation in food risks observed are often driven by origin of the product (i.e. domestic vs. imported); population preferences (e.g. food preferences and personal behaviours); climate variability (e.g. warming waters and seafood; genotypic distribution of the virus); food-processing methods (e.g. hand prepared vs machine or robotics-prepared); among other factors. It is important to note that the foods ranked to date are those for which a significant number of outbreaks have been reported, and/or those that have been well studied. However, risks can emerge in other foods with the same or similar characteristics.

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4

Hepatitis A virus

4.1. INTRODUCTION

Hepatitis A is a vaccine-preventable infection caused by the hepatitis A virus (HAV). The hepatitis A virus causes inflammation of the liver and is a major cause of acute hepatitis globally. Hepatitis, including that caused by HAV, is a notifiable/reportable disease in many countries. The average incubation period is 28 days. There is no specific treatment for infection with HAV. Transmission is mainly through the fecal-oral route, through direct contact with an infectious case or through contaminated food or water. Foodborne transmission of HAV is recognized as a serious public health problem and has been found to be equally important to person-to-person transmission in many subregions of the world (Havelaar *et al.*, 2015; WHO, 2015; FAO and WHO, 2008). Severity of infection is generally age dependent. Young children usually have asymptomatic infections, while adults are more likely to have mild to severe illness, with severity usually increasing with age. Fulminant hepatitis can occur in a small proportion of cases and is frequently fatal.

Hepatitis A virus distribution varies globally, with regions that can be classified as having either high, medium (intermediate), low or very low endemicity (WHO, 2022). Regions with poor sanitary and socioeconomic conditions generally have high endemicity, where most transmission is person-to-person and > 90 percent are infected before the age of 10 (WHO, 2022). In regions with medium endemicity, person-to-person transmission is important, and food and waterborne outbreaks occur. Regions with low or very low endemicity have low population immunity, which means a higher susceptibility to infection. Hepatitis A cases are more frequently reported in adults in regions with low or very low endemicity.

These regions tend to be more affluent with a higher standard of living. Infections in these regions are likely to be associated with travel to, and/or consumption of food from a high endemicity region. Infection may also occur through drug use, in people experiencing homelessness, and in men who have sex with men (Van Damme *et al.*, 2023). Poor sanitation and hygiene increase the risk of infection in susceptible populations.

Hepatitis A virus causes more than 159 million infections a year globally, with 39 000 deaths according to the World Health Organization (WHO) (WHO, 2022; IHME, 2019). Approximately 14 million cases and 27 000 deaths are attributed to foodborne transmission (Kirk *et al.*, 2015). The proportion of HAV cases attributed to food is estimated at 40 percent (Havelaar *et al.*, 2015; WHO 2015); 30 percent for Europe (Severi *et al.*, 2023). The United States Centers for Disease Control (CDC) estimates that in the United States of America, 41 percent of HAV cases are due to international travel while 7 percent are domestic foodborne cases (Scallan *et al.*, 2011). For HAV outbreaks in regions with low population immunity, and which are related to contaminated food, the food has been imported from intermediate or high endemic areas. Foodborne outbreaks with ongoing transmission, via person-to-person contact occurring weeks or months later, are also frequently reported (FAO and WHO, 2008).

Hepatitis A virus is a non-enveloped, icosahedral, single-stranded positive-sense RNA virus (27–32 nm diameter; 7.5 kb genome size) belonging to the genus *Hepatovirus* of the family *Picornaviridae* (McKnight and Lemon, 2018). Nine HAV species (A–I) have been identified as infecting animals including primates, several small mammals, seals, and marsupials. Human HAV belongs to the *Hepatovirus A* species. Hepatitis A virus has one serotype and six genotypes (three that infect humans). Genotypes are defined according to a sequence variability of the VP1 region of at least 15 percent while sub-genotypes differ by 7.0–7.5 percent (Van Damme *et al.*, 2023). Genotypes I–III (and subtypes IA, IB, IIA, IIB, IIIA, and IIIB) can infect humans (WHO, 2019). Genotypes I and III tend to be widely distributed and are the most prevalent genotypes identified. Subtype IA is responsible for most hepatitis A cases worldwide; subtype IB has been mainly found in the Mediterranean region and Europe, with IIIA being very common in Asia (Nainan *et al.*, 2006; Gholizadeh *et al.*, 2023). The distribution of the genotype, along with sequence data, can aid in tracking the geographic origin of strains (D'Andrea *et al.*, 2015; Kroneman *et al.*, 2018).

4.2. PREVALENCE IN FOODS

Foods can become contaminated with HAV at any point in the food chain. Contamination can occur during primary production (e.g. contaminated production waters [produce and bivalve molluscan shellfish] growing waters), harvest, and processing (e.g. human contact by pickers and packers), or by infected food handlers. Foods most frequently implicated with hepatitis A cases include bivalve molluscan shellfish, fresh and frozen berries, and prepared foods with extensive human handling. Other implicated produce items include most recently dates, pomegranate arils (seeds) (Table 3) and before then, semi-dried tomatoes (2009–2011).

TABLE 3 Foods associated with hepatitis A outbreaks in the last decade (2013–2023)

FOOD CATEGORY	OUTBREAK LOCATION	CASES	YEAR	RAW MATERIAL (COUNTRY)	LIKELY SOURCE OF CONTAMINATION	DETECTION IN FOOD	REFERENCE
Frozen pomegranate arils	United States of America	165	2013	Türkiye		Not detected	Collier *et al.*, 2014
	Australia	30	2018	Egypt	Pre-import processing	Detected in three samples	Franklin *et al.*, 2019
Fresh dates	Sweden	27	2018	Iran (Islamic Republic of)			Statens Serum Institute, 2018
	United Kingdom (England and Wales)	31	2021	Jordan		Detected in two samples	Garcia Vilaplana *et al.*, 2021
Imported Medjool dates	Australia	6	2021	Jordan	Not identified. Suspected contamination prior to or at packing.	Two out of ten samples /none by further sequencing	O'Neill *et al.*, 2022
Fresh blackberries	United States of America	16	2019	Mexico		Not detected	McClure *et al.*, 2022
Frozen mix berries	Several European countries	1 589	2013–2014	Bulgaria, Poland			Severi *et al.*, 2015
	Ireland	21	2013	Imported (not specified)		None out of 16	Fitzgerald *et al.*, 2014

TABLE 3 Foods associated with hepatitis A outbreaks in the last decade (2013–2023) (cont.)

FOOD CATEGORY	OUTBREAK LOCATION	CASES	YEAR	RAW MATERIAL (COUNTRY)	LIKELY SOURCE OF CONTAMINATION	DETECTION IN FOOD	REFERENCE
Frozen mix berries	Italy	1 803	2013–2014	10 different countries	Unknown	15 out of 1 982 1 sample sequenced	Scavia *et al.*, 2017
	New Zealand	7	2015	Imported	Suspected contamination prior to or at packing. Retail consumption		Lopez *et al.*, 2016
	New Zealand	35 (in 2022)	2022–2023	Imported	Suspected contamination prior to or at packing. Retail consumption		Horn *et al.*, 2023
	United States of America	162	2013	Türkiye			Collier *et al.*, 2014
Frozen strawberries	Denmark, Finland, Norway, and Sweden	103	2013			Negative	Lassen S.G. *et al.*, 2013
	Sweden, Austria	36	2018	Poland		Positive and sequence obtained	Enkirch *et al.*, 2018
	Germany	65	2018–2020	Egypt		None out of 3	Ruscher *et al.*, 2020
Mussels	Netherlands (Kingdom of the)	9	2012	United Kingdom	Growing waters	Samples not available	Boxman *et al.*, 2016
Scallops	Hawaii, United States of America	292	2016	Philippines		Positive (4 genomic copies per 1.7 g of scallops). Sequence obtained	Viray *et al.*, 2019
Salted clams	Republic of Korea	31	2019	Republic of Korea		Yes, sequences obtained	Hyun *et al.*, 2022
Oysters	China	110	2020	China		1 out of 20	Yan *et al.*, 2022

Sources: See References.

Hepatitus A virus prevalence in foods varies by region/country and is primarily impacted by endemicity status. Published monitoring studies focus on food types such as leafy greens, berries and bivalve molluscan shellfish that are frequently associated with reported outbreaks (Tables 4 and 5). There is an overall lack of data for other foods, including prepared and RTE foods (Food Safety News, 2020; Schenkel *et al.*, 2006; Hernández *et al.*, 2019). Outbreaks have occurred with some frequency at restaurants around the world, most notably in higher income countries, e.g. outbreaks associated with local bakeries in Germany and Spain, yet unfortunately, these are not often reported in the literature.

Most prevalence studies originate from regions of low or intermediate endemicity but may include domestic and/or imported food products (WHO, 2019; Jacobsen, 2018). A low HAV prevalence in foods is generally reported from regions of low or intermediate endemicity. For example, a prevalence of HAV of < 2 percent was reported in leafy salads and fresh berries collected at retail in Australia in 2013–14 (Torok *et al.*, 2019). Low prevalence or absence of HAV in berries has been reported in other countries (e.g. United States of America, 0.6 percent; Ireland, 2 percent; Argentina, 0 percent). Studies from Egypt (a high-endemicity country) show that HAV was frequently detected in fresh produce (27 percent, 13/48), green leafy salads such as watercress (31.2 percent, 31/48) and fresh strawberries (48 percent, 13/48) collected from two regions in 2019–2020 (Shaheen *et al.*, 2022; Elmahdy *et al.*, 2022). Testing for HAV RNA in foods associated with outbreaks is not always successful. This is due, in part, to the relatively long incubation period (i.e. the time between consumption and symptoms), lack of product to test, and challenges associated with often low contamination levels and virus recovery from complex matrices.

4.3. CONTAMINATION LEVELS IN FOODS

Data on HAV contamination levels in foods are available on those associated with outbreaks or cases, or from monitoring studies. Due to the long incubation period of HAV, data on foods associated with hepatitis A cases are generally limited to products with a longer shelf-life such as frozen bivalve molluscan shellfish, frozen foods, and produce with a shelf-life longer than four weeks. Hepatitis A virus contamination levels determined from foods as a result of monitoring studies does include a wider range of products, but studies are limited. Reported contamination levels of HAV do vary by food. Bivalve molluscan shellfish can bioaccumulate viruses present in contaminated growing waters, and so shellfish can contain moderately high contamination levels (10^2 to 10^4 genome copies/g digestive tissue) compared to the surrounding growing waters (Table 4).

TABLE 4 Prevalence and levels of hepatitis A virus in bivalve molluscan shellfish in samples collected last decade (2013–2023)

HAV COUNTRY ENDEMICITY	TYPE OF SHELLFISH	COUNTRY	SAMPLING PERIOD	NUMBER OF SAMPLES	POINT OF SAMPLING	PERCENT POSITIVE (%)	LEVELS	SAMPLES ASSOCIATED TO AN OUTBREAK	REFERENCES
Intermediate	Oysters and coquina clams	Vietnam	2015–2016	121	Dispatch centre	1.7	1.3×10^2 gc/g	No	Suffredini *et al.*, 2020
	Mussels	Türkiye	2014–2015	736	Production area	3.3	ND	No	Yilmaz *et al.*, 2018
Low	Oysters	Australia	2014–2015	297	Production area	No HAV detection			Torok *et al.*, 2018
	Mussels and clams	Italy	2015	352	Production area	21.9	LOQ to 5×10^3 gc/g DT	Samples collected after a contamination event	Suffredini *et al.*, 2017
	Mussels, oysters and clams	Italy	2013–2015	253	Production area (Class A and B)*	No HAV detection	-		La Bella *et al.*, 2017
	Mussels and clams	Italy	2015–2017	289	Production area (Class A and B)*	8.9	< LOQ to 4.2×10^2 gc/g		Fusco *et al.*, 2019
	Mussels, oysters and clams	Italy	2017–2019	162	Production areas (Class B), depuration and dispatch centre, restaurants and retail stores	0.6			Macaluso *et al.*, 2021

TABLE 4 Prevalence and levels of hepatitis A virus in bivalve molluscan shellfish in samples collected last decade (2013–2023) (cont.)

HAV COUNTRY ENDEMICITY	TYPE OF SHELLFISH	COUNTRY	SAMPLING PERIOD	NUMBER OF SAMPLES	POINT OF SAMPLING	PERCENT POSITIVE (%)	LEVELS	SAMPLES ASSOCIATED TO AN OUTBREAK	REFERENCES
Low	mussels, clams, cockles and oysters	Italy	2014–2019	2 266	Production areas	0.26			Pavoni *et al.*, 2022
	Oysters and mussels	Netherlands (Kingdom of the)	2013–2017	756	Production areas, dispatch centres and retail stores	0.16	3.70 $\log_{10}$ gc/g n = 1, mussel, production area	no	Dirks *et al.*, 2021
	Frozen scallops	Canada	1 April 2020 to 31 March 2022	229	Imported	0 (0/229)			CFIA, 2023

Notes: GC: Genome copies; DT: digestive tissue; ND: not determined
* EU regulations No. 627/2019 classified shellfish harvesting areas into A, B or C category on the basis of *Escherichia coli* levels in 100 g flesh and intravalvular fluid as follows: A (≤ 230 cfu *E. coli* in 80 percent of the samples and < 700 for the remaining 20 percent), B (< 4 600 *E. coli* for at least 90 percent of the samples, and < 46 000 *E. coli* for the remaining 10 percent), C (below 46 000 cfu *E. coli*).

Sources: See References.

TABLE 5 Prevalence and levels of hepatitis A virus in produce harvested in the last decade (2013–2023)

HAV COUNTRY ENDEMICITY	TYPE OF PRODUCE	COUNTRY	SAMPLING PERIOD	NUMBER OF SAMPLES	POINT OF SAMPLING	PREVALENCE (%)	LEVELS	REFERENCES
High	Green onions and lettuce	Egypt	2019–2020	48	Harvesting points	20.8 (10/48)	1.2×10^2 to 4.5×10^6 GC/g	Shaheen *et al.*, 2022
	Leafy greens and berries	Egypt	2019–2020	240	Harvesting points and retail	32.2 (78/240)	2.2×10^3 to 3.4×10^4 GC/g	Elmahdy *et al.*, 2022
Intermediate	Berries	Argentina	2016–2018 and 2020	184	Production plants and retail	0 (0/184)	NA	Oteiza *et al.*, 2022
	Vegetables	Czechia	2012–2016	623	Production plants and retail	0 (0/623)	ND	Huvarova *et al.*, 2021
Low	Domestic berries	United States of America	2022–2023	527		0.6 (3/527)	ND	U.S. FDA, 2024
	Imported berries			995		0.6 (6/995)	ND	U.S. FDA, 2024
	Cabbage, cucumber, lettuce and strawberries	Republic of Korea	2016–2017	535		0.2 (1/535)	ND	Shin *et al.*, 2019
	Leafy greens and berries			Australia	2013–2014	0 (0/302)	NA	Torok *et al.*, 2019
	Berries*	Several countries**	2009–2016	2 015		0.1 (2/2015)	ND	Li *et al.*, 2018

TABLE 5 Prevalence and levels of hepatitis A virus in produce harvested in the last decade (2013–2023) (cont.)

HAV COUNTRY ENDEMICITY	TYPE OF PRODUCE	COUNTRY	SAMPLING PERIOD	NUMBER OF SAMPLES	POINT OF SAMPLING	PREVALENCE, %	LEVELS	REFERENCES
Low	Root vegetables	Republic of Korea	Not specified	440	Retail and online markets	4.6 (20/440)	ND	Park *et al.*, 2023
	Bagged RTE vegetables	Italy	2014–2017	911	Retail	2. 6 (18/911)	ND	Terio *et al.*, 2017
	Berries	Ireland	2018	239	Retail	2.1 (5/239)	0.42 gc/g	Bennett *et al.*, 2023
	Cranberries	Canada	2021	234	Different producers	0 (0/234)	NA	Chatonnat *et al.*, 2023
	Berries and leafy greens		2014–2019	3 152		0.1 (3/3152)	ND	Pavoni *et al.*, 2022

Notes: * Mostly frozen, but also dried, in puree, syrup and as part of a finished product
** Bulgaria, Czechia, France, Germany, Poland, Russian Federation, Spain, Switzerland, Türkiye, and United States of America.
NA: not applicable, ND: not determined

Sources: See References.

Levels in bivalve molluscan shellfish are generally higher than in foods such as salads, berries and fresh produce, where contamination levels are seldom reported, often being less than the limit of quantitation (LOQ)/limit of detection (LOD) e.g. < 1 to approx. 10–20 genome copies per gram for fresh produce and berries, as shown in Tables 2 and 3 (Shaheen *et al.*, 2022; Elmahdy *et al.*, 2022). There is a lack of data on HAV contamination levels in other foods, particularly those that may become contaminated through food handlers during food preparation or serving. Low HAV levels in foods can make laboratory virus recovery and detection difficult. In Chapter 8, this report discusses the International Standard ISO 15216, which reports the LOD and LOQ for berries to be of approximately 4 and 10 genome copies/g, respectively, and for lettuce, approximately 3 and 32 genome copies/g, respectively. There are no data reported for other foods (Lowther *et al.*, 2019). The reported LOD and LOQ values are higher when using an adaptation of the ISO 15216 method for multicomponent foods (Hennechart-Collette *et al.*, 2021).

4.4. CONTAMINATION LEVELS IN ENVIRONMENT AND SURVIVABILITY (IN REGARD TO POTENTIAL TO CONTAMINATE)

Hepatitis A virus (HAV) is shed in feces at high contamination levels (10^3–10^{11} genome copies/g feces) (Costafreda, Bosch and Pintó, 2006; Kamel *et al.*, 2011), and viral RNA can be readily detected in influent wastewater when present in the community. HAV detection frequency in wastewater ranges from < 10 percent to 60 percent, with decreases noted over time in some regions in Europe (Bisseux *et al.*, 2018; Cuevas-Ferrando *et al.*, 2022; Fantilli *et al.*, 2023; Pellegrinelli *et al.*, 2019; Rodriguez-Manzano *et al.*, 2010). Levels are typically around 10^6 genome copies/L in regions where HAV has intermediate to high endemicity (Ouardani *et al.*, 2015; Prado *et al.*, 2021; Rachida and Taylor, 2020; Takuissu *et al.*, 2023). Receiving waters and other waters impacted by fecal contamination can therefore contain HAV. A systematic review and meta-analysis of 200 prevalence data from 144 articles over 34 years (1986–2020) calculated an overall prevalence of HAV of 16.7 percent (95 percent CI:13.4–20.3) in water (Takuissu *et al.*, 2023). While this figure includes 2 840 untreated wastewaters from 56 studies (HAV prevalence 31.4 percent), HAV was detected in 18 percent of treated wastewater samples, 15 percent of surface waters, and 2.3 percent of groundwater samples. As expected, prevalence was generally highest in countries with high endemicity (i.e. in Africa and in the eastern Mediterranean), and with lower prevalence (total < 10 percent) in countries with low endemicity, such as the United States of America (8.5 percent overall, 10.6 percent surface water). However, exceptions were noted, for example,

a 50 percent prevalence in groundwater in the United States of America (Takuissu *et al.*, 2023). HAV frequency and contamination levels would be expected to decrease in wastewater over time as public health improves. Most persistence and inactivation data on HAV has been determined from studies using laboratory-adapted strains (Sánchez, 2015).

Hepatitis A virus has been shown to retain its infectivity for extended periods in the environment including in wastewater. Contamination of irrigation waters or shellfish growing waters with HAV-containing wastewater can lead to the contamination of produce (Kokkinos *et al.*, 2017) or shellfish. As with other enteric viruses, HAV persists well in foods for several days on fresh produce, and for months, probably years, when frozen (Sewlikar and D'Souza, 2017; Cook *et al.*, 2018). Persistence for extended times on contact surfaces and hands has also been documented (Cook *et al.*, 2018).

Inactivating HAV while retaining food quality characteristics can be challenging. HAV can withstand mild pasteurization that would inactivate or control bacterial pathogens. While HAV can be efficiently inactivated by boiling, this is not acceptable for many foods. Alternative strategies for preventing initial contamination and acceptable virus inactivation methods without changing the properties of the food are needed. Reduction of infectious viruses on hard surfaces can be achieved following treatment with bleach and other sodium hypochlorite disinfectants, but only at very high contamination levels of 1 000–5 000 ppm available chlorine (CDC, 2023), and its efficacy is highly impacted by the presence of food and organic matter. These contamination levels are neither practical nor often are they even allowable in food production, processing and preparation.

4.5. SPECIFIC DETECTION METHODS AND MOLECULAR TYPING

Hepatitis A cases are laboratory diagnosed using serology (presence of HAV IgM or seroconversion) and/or detection of HAV nucleic acid, usually in blood. Sequencing of HAV from blood or feces may be used for genotyping and for surveillance purposes, including linking foodborne cases and investigating outbreaks (Boxman *et al.*, 2016). Virus contamination levels from foods, water and environmental samples are usually required prior to molecular analysis. Methods developed by ISO and the U.S. FDA remain the methods in predominant use for some foods (ISO 15216-1:2017 applies to bivalve molluscan shellfish, soft fruit, leaf, stem and bulb vegetables, and bottled water; U.S. FDA applies to bivalve molluscan shellfish, leafy greens, green onion, soft fruits, scallops, and finfish).

Due to challenges associated with the culture of wild-type HAV (Kanda *et al.*, 2020), cell culture that would inform on infectivity is not suitable for detection purposes. However, cell culture-adapted strains such as HM-175 18f are frequently used in laboratory-based studies on persistence and resistance.

While RT-qPCR assays remain the predominant method for detecting HAV (for example as described by Costafreda, Bosch and Pintó, 2006), the technology has seen much recent innovation. Commercial kits are much more widely available since the last FAO virus report (FAO and WHO, 2008). Ongoing innovation continues with the development of loop-mediated isothermal amplification (LAMP), digital/digital droplet PCR, and clustered regularly interspaced short palindromic repeats (CRISPR)-based methods. One of the limitations of conventional molecular-based methods is a lack of information on whether the nucleic acids detected represent an infectious virus or not. This can be overcome to some extent by using capsid integrity / dye-based methods. Unfortunately, these methods are less sensitive, and their suitability depends on the food matrix and food-processing technology used. Some techniques needed to prepare a food sample for subsequent PCR analysis (such as the ISO 15216 method) will damage the capsid, rendering capsid integrity methods unsuitable for viability assessment. This remains a research area in active development.

Where available, sequencing of both clinical samples and (when levels allow) foods is used for genotyping. There are distinct geographical distributions of HAV genotypes, so even partial genomic sequencing can aid in source identification during outbreak investigations (Nainan *et al.*, 2005). Partial sequencing (e.g. VP1-2A or entire VP1 gene region) (Robertson *et al.*, 1992; Costa Mattioli *et al.*, 2002; RIVM, 2023; Kroneman *et al.*, 2018; Yan *et al.*, 2022) is the most common approach used. Whole genome sequencing can provide higher resolution and distinguish closely related sequences but does require more resources in terms of technical and bioinformatic support. Sequencing may be used for genotyping, determining phylogeographic relationships or in epidemiologic surveillance, and for investigating foodborne outbreaks (Lemon *et al.*, 2018; Probert *et al.*, 2019). For partial sequencing, there is a need for a consensus method to assess the genotypic relationship of global HAV strains. Whole genome sequencing, genotyping and molecular diagnosis of HAV in human clinical samples have benefits for global epidemiology (Cleary *et al.*, 2023), but their application to food samples is currently quite limited due to the low virus contamination levels (RIVM, 2023; Kroneman *et al.*, 2018).

Host susceptibility and pathogenesis

The incubation period of HAV is approximately 28 days (range 15 to 50 days). Once a person is infected with HAV, the virus is shed in feces two weeks before the development of symptoms. Virus shedding continues for several weeks after symptom onset. Children and infants shed HAV for longer periods compared to adults (Chiriaco' *et al.*, 1986; Gholizadeh *et al.*, 2023; Phan and Hollinger, 2013; Tassopoulos *et al.*, 1986). Lifelong immunity following infection occurs. There is no known zoonotic transmission of HAV.

Haas and Eisenberg (2011) developed an HAV dose-response model using data obtained from Ward *et al.* (1958), for which the doses were measured as grams of feces from an infected individual. The ID_{50} (dose needed to infect 50 percent of those exposed) was estimated to be 0.5 g of feces, which is approximately 2 400 virus particles (Weir, 2020).

The pathogenesis of HAV includes the migration of the virus from the mucosa of the small intestinal wall to the liver via the portal vein within a few hours of ingestion, and its eventual replication in hepatocytes. HAV is then transported back to the gastrointestinal tract through bile. HAV is often detected at very high levels in the stool of infected persons (Bhilegaonkar and Kolhe, 2023).

Viral load is higher in persons presenting with jaundice during the early stages of viremia, and during this phase HAV can also be detected in blood samples. HAV infection may induce jaundice or not. In adults, anicteric or icteric hepatitis is commonly observed whereas, in children, infection is mostly subclinical. Symptoms may recur in up to 10 percent of patients after recovery, causing the disease to last for weeks or months. Liver damage can be severe, especially in adults who lack protective antibodies acquired during childhood or vaccination. Hepatic failure resulting in death is occasionally seen in patients above 50 years of age (Bhilegaonkar and Kolhe, 2023; Gholizadeh *et al.*, 2023; Lai and Chopra, 2023; Phan and Hollinger, 2013).

While there is no clear seasonality, HAV infection may be associated with the availability of seasonal fresh foods and/or contamination risks changing over seasons/climate/weather events. Based on outbreak data, the ages affected by foodborne transmission of HAV depend on susceptibility and food preferences.

4.6. REGIONAL ISSUES/DIFFERENCES

The incidence of hepatitis A and disease burden varies considerably between regions depending on the ssociodemographic index (Zeng *et al.*, 2021). Within countries, there may also be disproportion in infections in certain populations. Globally, there are certain factors that influence the incidence of disease, i.e. increased conflict, refugee movement, and regional disasters, including catastrophic weather events as may occur due to global climate change. These place pressure on infrastructure and put people in closer proximity with associated sanitation issues. The result is more disease transmission, including via foodborne routes. This will, of course, vary by region, population, and even country. Examples would include increased risk of shellfish contamination from wastewater and storm overflows (Younger, Kershaw and Campos, 2022) or increases in homelessness with associated spikes in HAV incidence (Foster *et al.*, 2018).

Low- and middle-income countries have the greatest hepatitis A burden, with most cases and deaths reported in the WHO regions of Southeast Asia, Africa and the eastern Mediterranean (Jacobsen *et al.*, 2018; Lemon *et al.*, 2018). The high burden of HAV in countries with poor hygiene standards and low socioeconomic conditions means that people are exposed to the virus early in life, resulting in frequent asymptomatic infections and a high proportion of immune adults (HAV infection gives lifelong acquired immunity) (Migueres, Lhomme and Izopet, 2021). The global epidemiology of HAV has been changing over the past few decades, with the general trend of a decreasing incidence rate (Jacobsen *et al.*, 2018; Lemon *et al.*, 2018). This decrease has been mainly due to improvements in sanitation, better access to clean water, and vaccinations, meaning fewer infections in young children. Paradoxically, with a decreasing endemicity, the incidence of infections in older adults increases, along with the number of symptomatic infections and associated disease severity (WHO, 2022; Migueres, Lhomme and Izopet, 2021).

Globalization of the food supply, along with increasing global travel, however, means that countries that have no or low HAV incidence, and with a susceptible population, have greater exposure, particularly of the higher risk populations such as older adults. For food, consumption patterns in certain regions may allow increased transmission to consumers (e.g. populations consuming healthy, raw or less processed foods and "out of season" foods such as fresh berry fruits now available year-round). The trend towards more consumption of prepared foods and increases in shellfish or fruit consumption may also impact the risk of exposure and illness. Foodborne outbreaks may be multicountry/region and may be difficult to identify. Sequence characterization and data sharing globally may assist in identifying linkages between otherwise unrelated cases, if the appropriate infrastructural changes can be made.

The widespread availability and adoption of hepatitis A vaccines as part of the national vaccination schedules will influence incidence within a given country. For example, numbers of reported cases declined substantially in the United States of America following the inclusion (albeit optional in some states) of HAV immunization in the national childhood vaccination schedule, with infection rates shown to decrease more than 95 percent since 1995 (when the vaccine first became available), despite uneven coverage (Murphy *et al.*, 2016). Vaccines may also be used for persons at heightened medical risk if infected, for travel to a region in which HAV is endemic, for persons with occupational or exposure risk, in response to an outbreak, or as a proactive control measure in food handlers (Fallucca *et al.*, 2023).

Finally, the relationship between government and industry partners may lead to different decisions in terms of surveillance and management strategies, potentially leading to variations in prevalence estimates (or even data availability) or shifts in management priorities due to industry or regulatory pressures.

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5

Hepatitis E virus

5.1. INTRODUCTION

Hepatitis E is an important disease in many developing countries where it is epidemic, but it is also endemic in many industrialized countries. According to the WHO, an estimated 20 million people worldwide are infected by hepatitis E virus (HEV) each year via contaminated water, food, or other vehicles, leading to 3.3 million cases of hepatitis E and 44 000 hepatitis E-related deaths (WHO, 2023). There is no global estimation of cases that are attributed to food. For Europe, where foodborne transmission of HEV appears to be a major route, 21 000 acute clinical cases with 28 fatalities were notified between 2005 and 2015 (EFSA, 2017). This number of notified cases has increased from 514 cases per year in 2005 to 5 617 cases in 2015 (Aspinall *et al.*, 2017). However, hepatitis E is not notifiable in all European countries, and it is still considered to be an underdiagnosed disease in Europe (EFSA, 2017) and likely in other regions of the world as well. Data on numbers of foodborne HEV infections for other countries and world regions are even more scarce, and in some countries HEV is not often considered in travel-related cases of hepatitis.

The hepatitis E virus (HEV) is an emerging virus that is classified in the family *Hepeviridae* (Purdy *et al.*, 2022). Within the subfamily *Orthohepevirinae*, the species *Paslahepevirus balayani* contains the majority of the human-infecting hepatitis E virus genotypes, including HEV genotypes 1, 2, 3, 4 and 7. HEV is a non-enveloped, spherical virus particle of approximately 30–35 nm in diameter when shed in feces, although the virus exists in a quasi-enveloped form in circulating blood and cell culture supernatant. The genome of HEV is a single-stranded positive sense RNA,

approximately 7.2 kb in size, which consists of three partially overlapping open reading frames (ORFs) encoding a multifunctional non-structural polyprotein (ORF1), a capsid protein (ORF2), and a small phosphoprotein (ORF3) that is involved in virus replication.

5.2. PREVALENCE IN FOODS

Foodborne hepatitis E cases are caused mainly by HEV-3 and, to a lesser extent, HEV-4. The HEV prevalence in food varies depending on the types of food and geographic origins. The main source of HEV is from domestic pigs, but game animals such as wild boar, deer and rabbit are also sources of HEV (Pavio *et al.*, 2017). A recent review estimated that, at the global level, nearly 60 percent of domestic pigs and 27 percent of wild boars have encountered HEV infection based on seroprevalence rates (Li *et al.*, 2022). Nearly 13 percent of domestic pigs and 9.5 percent of wild boars were actively infected based on HEV RNA positivity, and about 10 percent of commercial pork products were positive for HEV RNA (Li *et al.*, 2022).

Liver from infected animals is the main source of HEV, although blood – and to a lesser extent skeletal muscles – can also contain HEV. Based on worldwide reports, the HEV RNA detection rates in pig liver ranged from 0 to 21 percent; the majority of studies reported detection rates between 2 and 8 percent (Pavio *et al.*, 2017). HEV RNA was detected in the blood of 6.3 percent of market-weight pigs from 25 slaughterhouses in ten states in the United States of America (Sooryanarain *et al.*, 2020), but this may be dependent on region and age as much higher percentages have been detected in studies in the Kingdom of the Netherlands (Boxman *et al.*, 2022; Meester *et al.*, 2022). In pig muscle tissue, the HEV RNA detection rates ranged from 0 to 6 percent (Pavio *et al.*, 2017). Undercooked or raw sausage containing blood and liver as well as liver paté have been frequently found to be contaminated by HEV. HEV RNA detection rates between 16 percent and 47 percent have been described worldwide for sausages or meat products containing pig liver (Pavio *et al.*, 2017). HEV RNA was also detected with lower rates in pork products not containing added liver (e.g. raw pork sausages, cured meat sausages), possibly due to use of diaphragm muscle contaminated with liver remains (Szabo *et al.*, 2015; Boxman *et al.*, 2020), and chitterlings.

Additionally, shellfish raised in contaminated seawater has been repeatedly reported to contain HEV (Treagus *et al.*, 2021). A hepatitis E outbreak on a cruise ship has been linked to consumption of shellfish while on board (Said *et al.*, 2009), although the relevance and extent of shellfish in contributing to foodborne

human hepatitis E is currently not well understood. There are limited data on HEV contamination in fresh and frozen produce like herbs, lettuce and berries (Treagus *et al.*, 2021). HEV detection in raw milk has also been described, mainly for HEV-4 in China, but more studies are needed to definitively assess the risk of raw milk for HEV transmission (Santos Silva *et al.*, 2022). Sewage or water contaminated with HEV (including HEV-1 and HEV-2 in the respective geographic areas, see 5.7) may also be a source for food contamination through irrigation or growing waters for shellfish.

5.3. CONTAMINATION LEVELS IN FOODS

The contamination levels of HEV vary from food to food. Generally, liver from pig and game animals has the highest contamination levels of HEV. HEV genome contamination levels between 20 and 10^7 RNA GC[8] RNA copies/g have been reported for pig and wild boar liver, respectively (Pavio *et al.*, 2017). Blood also contains a relatively high amount of HEV, which can range from < 100 to 10^6 genome copies/ml serum as described for slaughtered market-weight pigs in the United States of America (Sooryanarain *et al.*, 2020). Virus contamination levels in skeletal muscles is generally lower, e.g. 500 and 4 000 genome copies/g, as have been reported for wild boar and deer muscle (Pavio *et al.*, 2017).

The HEV RNA contamination levels in liver-containing meat products ranged from 4 to 2 × 10^6 copies/g (Pavio *et al.*, 2017). A lower mean HEV RNA contamination level of 575 (2.76 $\log_{10}$) genome copies per 5 g has been reported for raw pork sausages without liver in a study from the Kingdom of the Netherlands (Boxman *et al.*, 2020). Limited data are available on HEV contamination levels in shellfish or other food products, but one study reported between < 100 and 10^5 HEV RNA copies/g of shellfish (Rivadulla *et al.*, 2019). Vegetable samples tested with the ISO method did not yield positives (Randazzo *et al.*, 2018).

5.4. CONTAMINATION LEVELS IN ENVIRONMENT AND SURVIVABILITY (IN REGARD TO POTENTIAL TO CONTAMINATE FOODS)

The hepatitis E virus (HEV) has been detected from numerous environmental sources including sewage and wastewater, river water, pig manure, irrigation water, drinking water, and seawater (Takuissu *et al.*, 2022). A recent systematic review and meta-analysis of 87 prevalence studies from 58 publications calculated an overall HEV RNA prevalence of 9.8 percent in water matrices.

Higher prevalence was reported in untreated wastewater (15 percent) compared to treated wastewater (3.8 percent) and drinking water (4.7 percent). Approximately 66 percent of these prevalence studies were performed in Europe (Takuissu *et al.*, 2022). In Germany, a median of up to 10^3 GC/100 ml was detected in effluent samples of wastewater treatment plants and up to 2×10^3 GC/100 ml in river water after combined sewer overflow (Beyer *et al.*, 2020). However, there is very limited knowledge on virus contamination levels in environmental waters (Cuevas Ferrando *et al.*, 2020).

HEV has been reported to be highly resistant to commonly used chemical, physical and environmental inactivation methods. Incubation of HEV at pH 2-9 for 3 h at room temperature did not lead to significant decrease in infectivity (Wolff *et al.*, 2020b). High salt concentrations, e.g. 20 percent NaCl with addition of 0.015 percent sodium nitrite or 0.03 percent sodium nitrate, did not significantly decrease infectivity of HEV (Wolff *et al.*, 2020a). In addition, infectious HEV could be detected after drying on different surfaces for up to four weeks storage at 23 °C and up to eight weeks storage at 4 °C (Wolff *et al.*, 2022).

Proper heating can inactivate HEV. It has been reported that HEV infectivity in experimentally contaminated pork liver pâté was abolished at 71 °C for 20 min (Barnaud *et al.*, 2012). However, medium to-raw cooking conditions do not completely inactivate HEV. For instance, incubation of HEV contaminated commercial pork liver homogenates at 56 °C for 1 h did not inactivate the virus, although HEV was inactivated by boiling for 5 min or stir-frying at 191 °C with an internal temperature of 71 °C for 5 min (Feagins *et al.*, 2008). Using a quantitative cell culture-based method, > 3.9 log decrease was determined for HEV in PBS by heating at 70 °C for 2 min (Johne *et al.*, 2016). D and z values can be estimated from published data but may vary widely between different experimental systems, e.g. T_{ref} 60 °C, D = 40.5 sec, z = 38 °C (Johne *et al.*, 2016) vs T_{ref} 60 °C, D = 9.3 min, z = 21 °C (Barnaud *et al.*, 2012). Generally, the thermal inactivation patterns of HEV appear to be similar to those of HAV (Bozkhurt, D'Souza and Davidson, 2015; Bozkhurt *et al.*, 2015).

5.5. SPECIFIC DETECTION METHODS AND MOLECULAR TYPING

Routine detection of infectious HEV from food samples is currently not established (Cook, D'Agostino and Johne, 2017). The few available cell culture systems are mostly inefficient or restricted to the propagation of a few culture-adapted HEV strains (see Chapter 8).

HEV detection in food or food products therefore relies mainly on molecular methods such as RT PCR, RT qPCR, RT-ddPCR, for which several sensitive and broadly reactive protocols have been published (e.g. Jothikumar *et al.*, 2006; Garson *et al.*, 2012). A WHO standard for HEV quantification by molecular assays has been established (PEI, 2018). For HEV RNA detection in distinct food matrices, several published protocols are available, and a few methods have been validated in interlaboratory ring trials, e.g. for pork liver or meat products like liver sausage (Althof *et al.*, 2019; Trojnar *et al.*, 2020). ISO methods for HEV detection in meat, liver, meat products and liver products are currently in development.

Molecular typing of HEV is usually performed by sequencing of a small fragment of the HEV genome, which can determine the HEV genotypes (e.g. HEV-3) and subtypes (e.g. 3a, 3c, 4d). Some of those typing/subtyping methods have been recently validated in an interlaboratory ring trial (Baylis *et al.*, 2021). A WHO reference panel of 11 HEV genotypes/subtypes is available (PEI, 2015). Additional strain characterization including sequencing of larger fragments and NGS technologies could be important for outbreak investigation. The interdisciplinary network HEVnet shares HEV molecular and epidemiological data (Mulder *et al.*, 2019).

5.6. HOST SUSCEPTIBILITY AND PATHOGENESIS

Since the discovery of the first animal strain of HEV from pigs in 1997 (Meng *et al.*, 1997), the host range of HEV has greatly expanded (Pallerla *et al.*, 2020). HEV-1 and HEV-2 are restricted to humans and are mainly transmitted via contaminated water. HEV-3 infects a wide range of animal species including domestic and wild pigs, deer, mongoose and humans. A distantly related HEV 3 subtype has been identified from rabbits and has also been reported in some human cases. HEV 4 mainly infects domestic and wild pigs as well as humans. HEV-7 infects dromedary camels with a single description of a human infection. Animals infected with HEV-3 or HEV-4 do not exhibit overt clinical disease, although microscopic lesions of hepatitis can be evident in HEV-3 infected pigs.

The pathogenesis of HEV in humans is not well understood (Yadav and Kenney, 2021). HEV can enter the human host through the gastrointestinal tract, via the fecal-oral route by contaminated food or water; infection can also occur via blood transfusion. Zoonotic transmission can occur through contact with infected animals. HEV replicates in small intestinal epithelial cells before entering the bloodstream and reaching the target organs, mainly the liver. The development of disease is dependent on several host and viral factors, with an involvement of the immune system (Yadav and Kenney, 2021).

The majority of HEV infections in humans are mild or subclinical. Symptomatic cases of hepatitis E typically have an incubation period between 15 and 60 days (Teshale, 2011). HEV-1 is associated with fulminant hepatic failure with increased mortality in infected pregnant women. A general mortality rate of up to 3 percent is described for young adults, whereas it may reach 30 percent in pregnant women infected with HEV-1 (Pallerla *et al.*, 2020).

HEV-3 and HEV-4 mainly cause acute hepatitis, which can be severe, predominantly in individuals with underlying conditions like pre-existing liver disease (Pallerla *et al.*, 2020). In addition, chronic HEV infections are increasingly diagnosed in people with immunosuppressive conditions (e.g. solid organ transplant recipients, HIV patients or those receiving chemotherapy), which can lead to life-threatening cirrhosis and liver damage (Takakusagi, Kakizaki and Takagi, 2023). HEV-3 is also associated with a number of neurological sequalae including neuralgic amyotrophy, Guillain Barré syndrome, myelitis, and encephalitis (Lhomme *et al.*, 2021). For example, 16.5 percent of HEV-infected patients in France (Abravanel *et al.*, 2018) and 30.4 percent of acute hepatitis E cases in Switzerland (Ripellino *et al.*, 2020) reported neurological symptoms. It appears that both the central nervous system and the peripheral nervous system can be affected.

5.7. REGIONAL ISSUES/DIFFERENCES

As noted throughout this report, regional differences are critical regarding virus surveillance and detection. For HEV, the genotypes are differently distributed in the regions of the world (Pallerla *et al.*, 2020). HEV 1 is mainly prevalent in Asia and Africa. HEV-2 has caused outbreaks in Mexico and several African countries. The circulation of the human-specific genotypes HEV-1 and HEV-2 can generally be associated with poor sanitation. Sporadic and clustered cases of HEV-3 infection have been reported in North and South America, Europe, Australia and New Zealand. HEV-4 is mainly prevalent in Southeast Asia, and some sporadic cases have also been reported in Europe. HEV-3 and HEV-4 infections can generally be connected with consumption of pork or pork products. HEV-7 has only been reported in the Near East so far in connection with dromedary camels. Travel-acquired sporadic cases of hepatitis E can often be traced back to the region of travel based on strain specifications.

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Rotavirus

Rotaviruses are naked double-stranded RNA viruses and belong to the family *Sedoreoviridae* and the genus Rotavirus (Matthijnssens *et al.*, 2022). The 100-nm non-enveloped virus particle is composed of three concentric protein layers and contains 11 segments of double-stranded RNA. The genome of rotavirus encodes for six structural and six non-structural proteins (Dian *et al.*, 2021). The two proteins defining the serotype are VP4 and VP7 also known as P and G proteins, respectively, and are located on the outer capsid (Zhao *et al.*, 2021). Rotaviruses of genotypes G1, G2, G3, G4, G9, and P[4] or P[8] are responsible for the highest disease burden in humans (Velasquez and Jiang, 2019). Thus far, ten species of rotavirus (A-J) have been recognized (Dian *et al.*, 2021). These ten groups are defined by the middle antigen, VP6 by which Group I was newly discovered in dogs and J in bats (Yandle *et al.*, 2020). However, the groups causing infection in humans are species A, B, C, and H (Dian *et al.*, 2021). Group A has been recognized as causing the highest disease burden in humans (Dian *et al.*, 2021), responsible for 90 percent of human rotavirus cases.

Rotavirus is an important cause of severe diarrheal illnesses, and a leading cause of severe, dehydrating gastroenteritis in children < 5 years of age. Rotavirus is reported to cause 528 000 deaths per year and around 2 million hospitalizations annually (Hallowell *et al.*, 2022). Rotaviruses are ubiquitous and infect almost every child globally by 3–5 years of age unless vaccinated. But although the prevalence of rotavirus infection in children hospitalized with diarrhea is similar worldwide, the use of rotavirus vaccination varies, and the children with fatal rotavirus infections tend to live in low-income countries (Crawford *et al.*, 2017). Out of 128 500 deaths related to rotavirus infections in 2016, 104 733 occurred in sub-Saharan Africa (Troeger *et al.*, 2018; Anonymous, 2022). Other than gastroenteritis, rotavirus is a prominent cause of seizures, hepatobiliary diseases, Type 1 diabetes, and respiratory illness (Dian *et al.*, 2021).

There are several approved vaccines against rotavirus (Varghese *et al.*, 2022). In December 2010, the WHO Strategic Advisory Group of Experts (SAGE) advised on the introduction of rotavirus vaccines in national immunization programmes worldwide (Varghese *et al.*, 2022). The numbers reported by WHO/UNICEF Estimates of National Immunization Coverage (WUENIC) on rotavirus vaccination coverage highlight an increase from 40 percent in 2019 to 51 percent in 2022 globally (WHO, 2022).

The primary site of rotavirus infection is the small intestinal villi, where virus replication mainly occurs in the cytoplasm of mature enterocytes (Amimo *et al.*, 2021). The incubation period for rotavirus A infection is estimated to be between 48 and 72 hours with the virus being at its greatest transmissibility within this period (Oh, Jeon and Kim, 2021). The infected cells produce viral enterotoxin NSP4 which attaches to intestinal epithelial cells inducing the secretion of chloride into the intestinal lumen (Amimo *et al.*, 2021). High concentrations of chloride ion cause an osmotic gradient that enhances the transmission of water into the intestinal lumen resulting in diarrhea (Amimo *et al.*, 2021).

While foodborne illness attributed to rotavirus is rare, there remain some areas of concern. The most common route for transmission is person to person. Foodborne rotavirus outbreaks have been reported, including an outbreak in a college in the United States of America associated with contaminated sandwiches (CDC, 2000) and an outbreak in a sanatorium in Germany associated with contaminated potato stew (Mayr *et al.*, 2009). In the United States of America, rotavirus is thought to cause less than 1 percent of foodborne illness (Painter *et al.*, 2013). There are no standard methods for the detection of rotavirus from foods, but some studies have been performed to investigate the presence of rotavirus in food. Viral RNA has been detected in berries, other fresh produce, bivalve molluscan shellfish, and meat (beef, chicken, and pork) (Oteiza *et al.*, 2022; Ito *et al.*, 2021; Soares *et al.*, 2022; Fusco *et al.*, 2017; Rafieepoor *et al.*, 2024). Although the vast majority of human rotavirus disease is caused by typical human-adapted rotavirus strains, zoonotic transmission may be possible in association with pigs and cattle, and perhaps other animal species (Díaz Alarcón, Liotta, and Miño, 2022; Martella *et al.*, 2010; Geletu, Usmae, and Bari, 2021). Reassorted rotaviruses with gene segments from both animal and human strains have been identified (Kamoto *et al.*, 2016). It would be advisable to better assess food samples for monitoring zoonotic transmission and strain evolution.

In the absence of standardized methods, various RNA extraction methods have been investigated for detecting rotavirus in food (Hatib *et al.*, 2021). Rotavirus RT-qPCR detection kits used in clinical settings may be combined with NA extraction methods as they are fast, sensitive and able to detect non-cultivatable

viruses (Hatib *et al.*, 2021). Examples of extraction and detection of rotavirus RNA in bivalve molluscan shellfish and berries are reported in the literature (do Nascimento *et al.*, 2022; Hoque *et al.*, 2022; Ito *et al.*, 2021; Yu *et al.*, 2023; Oteiza *et al.*, 2022). One study has been applied to meat samples such as beef, chicken, and pork (Soares *et al.*, 2022). Since rotavirus is a dsRNA virus, it may behave during extraction in a manner that differs from the more prevalent ssRNA viruses, but this has not been characterized to date. There is limited research on foodborne transmission of rotavirus and this remains a research gap.

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Other emerging viruses

The viruses included here are those that have the potential for foodborne transmission and can cause severe disease, but for which foodborne transmission is poorly characterized. For example, while the potential exists for enteric viruses like sapovirus, adenovirus and astrovirus to be transmitted via foods, the lack of epidemiological evidence makes this difficult to fully evaluate. These viruses are common causes of acute gastroenteritis around the world and have been associated with outbreaks in children (Luo *et al.*, 2021; Mousavi Nasab *et al.*, 2020).

Sapovirus is similar to norovirus and is a genus in the *Caliciviridae* family. Sapovirus is often associated with sporadic cases and outbreaks of acute diarrhea most often reported in children.

Human adenovirus is unique in that it is a non-enveloped double-stranded DNA virus that is grouped into seven genotypes and numerous serotypes, of which HAdV41 and HAdV40 are known as the enteric adenoviruses.

Astrovirus is a non-enveloped single-stranded RNA virus. Human astrovirus causes diarrhea in children, and some speculate it may be the major cause of childhood diarrhea (Vu *et al.*, 2017). Better regional and global surveillance is needed to determine the role of contaminated foods in transmission of these enteric viruses.

Viral tick-borne encephalitis, caused by an enveloped flavivirus, has occurred in Europe and is mainly transmitted by tick-bites. If the infected ticks bite animals like goats, when the animals develop viremia, the milk may become contaminated with the virus. Outbreaks of human encephalitis have been attributed to consumption of raw milk and unaged raw-milk cheeses (Buczek *et al.*, 2022). Mostly small outbreaks in eastern Europe have been recorded. This disease may be spreading across northern Europe in connection with changing temperatures and changes

in the geographical range of the vector (*Ixodes ricinus* and *Ixodes persulcatus*) (Voyiatzaki *et al.*, 2022). The encephalitis disease caused by this virus can be severe, leading to mortality rates of 0.5–2 percent of infections caused by the European virus subtype, but as high as 5 and 20 percent of infections caused by the Siberian and Far-Eastern subtypes, respectively (Buczek *et al.*, 2022). Although severe disease warrants concern, at this time foodborne infections remain relatively rare. Pasteurization of raw milk inactivates tick-borne encephalitis virus. Recently, detection methods for tick-borne encephalitis virus in milk and milk products have been developed (Müller *et al.*, 2023; Hennechart-Collette *et al.*, 2022).

Nipah virus is an enveloped zoonotic henipavirus transmitted through contact with infected animals (fruit bats or pigs), as well as food and materials contaminated with their saliva or urine. Outbreaks may have a high mortality rate in the Indo-Bangladesh regions. This virus of bat origin can cause infections in pigs and humans and has been associated with foodborne transmission. Nipah virus can be spread from person-to-person through bodily fluids (blood, urine, or saliva). An outbreak in Malaysia-Singapore was related to contact with pigs, and an outbreak in the Philippines was associated with horse slaughter; however, most other Indo-Bangladesh outbreaks were associated with consumption of raw date palm sap contaminated by fruit bats, with a high secondary attack rate (Banerjee, 2019). The case fatality rate for a 2023 outbreak related to the consumption of date palm sap in Bangladesh was 73 percent, according to a press release by the WHO (2023). The virus may potentially be transmitted through raw milk, but data are inconclusive. Person-to-person transmission of Nipah virus can occur after interaction with infected animals. Enhanced Nipah virus surveillance is warranted along with education from a One Health perspective.

Other viruses have the potential to initiate infection through the gut and may be excreted in feces, but epidemiological evidence does not necessarily support foodborne transmission. These include avian influenza and Middle East respiratory syndrome coronavirus (MERS-CoV), both enveloped viruses.

Middle East respiratory syndrome coronavirus (Accroding to the FAO style, we need to avoid beginning sentences with an abbreviation.) is a zoonotic virus that was linked to human infections via exposure to dromedary camels in the Near East, Africa, and South Asia. For avian influenza virus strains H5N1 and H5N8, zoonotic transmission can occur, although foodborne transmission is rare. Infections typically occur through very close contact with poultry raised in households, especially in areas where swine, poultry and humans live in close contact. Birds raised for cockfighting, which may have close contact with humans, may also be involved in transmission. Mammalian transmission of H5N1 has occurred and contact with these animals could impact human infections

(Agüero *et al.*, 2023; Huang *et al.*, 2023). At the time of writing this report, H5N1 infections had been reported in dairy cattle in several states of the United States of America,, resulting in one mild human case presumed to be of zoonotic origin (CDC, 2024). At this time, H5N1 manifests itself in cattle as a fairly mild illness but significantly reduces milk production (Burrough *et al.*, 2024). The spillover into dairy cattle has increased public concerns over the safety of the milk supply, particularly in the wake of recent detection of viral RNA fragments in commercial milk (Manas and Ward, 2024). The U.S. Food and Drug Administration and the U.S. Department of Agriculture have firmly supported the safety of commercially processed milk and stated the low risk of disease transmission through pasteurized milk and milk products, largely based on data on the sensitivity of earlier highly pathogenic avian influenza strains subjected to the less rigorous egg pasteurization process (Chmielewski *et al.*, 2013; Chmielewski, Beck and Swayne, 2013; U.S. FDA, 2024). There is potential risk associated with handling and consumption of raw milk from infected animals, restating the importance of pasteurization. Unpasteurized milk poses food safety risks from potential contamination by foodborne pathogenic bacteria.

At least three other viruses are on the radar for consideration: bovine leukosis virus, bovine polyomavirus, and circovirus. While these have not been identified as zoonotic viruses, there is some evidence that these viruses have been identified in cancerous tissues of humans (Gao *et al.*, 2022). Their potential for transmission in foods is unlikely, but unknown at this time.

Detection of all of the "emerging" viruses relies on techniques similar to detection for other foodborne viruses, but standardized methods do not yet exist. Since some of these emerging viruses of foodborne interest may be enveloped, they would be expected to be more sensitive to inactivation than are the typical non-enveloped enteric viruses, including food-processing methods and standard cleaning and sanitation techniques used in the food supply chain. Enhanced surveillance efforts are critical for all of these viruses and should be pursued in an effort to determine illness burden and identify novel transmission routes.

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8

Analytical methods and indicators

8.1. STANDARD METHODS FOR DETECTION OF FOODBORNE VIRUSES

Since the 2007 FAO/WHO expert consultation (FAO and WHO, 2008), standardized detection and quantification methods for enteric viruses in food have been developed, validated and published under the auspices of the European Committee for Standardization (CEN) Working Group on the Microbiology of the Food Chain. Their task was to develop a method for detecting HAV and human norovirus GI and GII in foodstuffs, based on RT qPCR. The matrices covered by the method were: bivalve molluscan shellfish, including oysters and mussels; soft fruit; leaf, stem, and bulb vegetables; bottled water; and food (preparation) surfaces. In 2013, the method was published as a technical specification (TS) by ISO, as ISO/TS 15216-1:2013 (EFSA, 2013). Thereafter, it was published as a validated ISO standard in two parts. Part 1 specifies a method for quantification (ISO 15216-1:2017; ISO 15216-1: 2017/Amd 1:2021). A qualitative (presence / absence) version was also published, describing the non-quantitative detection method ISO 15216-2: 2019 (ISO 15216-2:2019, 2019).

In brief, both parts of the method contain matrix-specific procedures for the recovery, and for some matrices, the contamination levels of viruses from samples. Following virus recovery, samples are then subjected to nucleic acid extraction by lysis with guanidine thiocyanate and adsorption to silica. Target sequences within the viral RNA are subsequently amplified and detected by RT-qPCR using

oligonucleotides targeting the ORF1/ORF2 region of norovirus GI or GII or the 5' non-coding region of the HAV genome. Both qualitative and quantitative parts of the method (ISO 15216-1:2017; ISO 15216 2:2019) prescribe the use of quality assurance controls, which are:

- a negative process control, i.e. target pathogen-free sample(s) of the food matrix, or target a pathogen-free non-matrix sample that is run through all stages of the analytical process;
- a positive extraction control, i.e. a virus (usually non-pathogenic and similar to the target virus) added to the sample portion at the earliest opportunity prior to virus extraction to determine extraction efficiency;
- a negative RNA extraction control(s), i.e. a control free of target RNA carried through all steps of the RNA extraction and detection procedure to monitor for potential contamination events;
- a negative RT-qPCR control, i.e. an aliquot of molecular grade water used in a RT-qPCR reaction to assess contamination in the reagents; and
- an amplification control, i.e. a reference RNA standard added to an aliquot of sample RNA, the amplification of which is used to assess for matrix-associated inhibition of RT-qPCR.

Quantification of target copies per microliter of sample RNA in Part 1 is done by reference to a standard curve generated from a dilution series of dsDNA carrying the relevant target sequence. Quantitative results are, notably, not corrected to account for the efficiency of the reverse transcription due to the use of dsDNA for quantification. Neither are they corrected for extraction efficiency or inhibition of the RT-qPCR. Both ISO 15216-1 and -2 describe criteria which the test results must meet:

- the minimum extraction efficiency, based on the RT-qPCR results for the process control virus seeded into the sample and run in parallel through all stages of the analytical process, as well as
- a minimum of amplification efficiency, based on RT-qPCR results for the target-specific RNA standard added to each sample.

Procedures for virus extraction were selected in part based on the ability to perform these methods in laboratories housing routine equipment and using general buffers and reagents. For nucleic acid extraction, an assay should give a purity of nucleic acids that is at least comparable to the purity obtained by silicon-binding technology (Boom *et al.*, 1990). The primer and hydrolysis probe sequences used for detection must be (i) located in the specific genome region identified in the ISO 15216, (ii) published in peer-reviewed journal literature, and (iii) verified using a broad range of target virus strains. These oligonucleotides, as well as the cycling

parameters, platforms, or extraction or detection kits, are given in informative annexes within the ISO 15216 standards. The user is advised to optimize these conditions before use.

The ISO 15216 is validated for a number of sample matrices, i.e. Pacific oysters and mussels as representatives of bivalve molluscan shellfish; raspberries as representative of soft fruit; lettuce and green onion as representative of leaf, stem, and bulb vegetables; bottled water; and pieces of bell pepper as examples for testing food (preparation) surfaces by swabbing (Lowther *et al.*, 2019). The method was not validated for detection of the norovirus GI and GII, or HAV RNA, in other foodstuffs (including multicomponent foodstuffs), or any other matrices. The quality assurance parameters, such as the extraction and amplification efficiency, may help to determine whether the ISO 15216 methods are appropriate for a broader set of matrices. Meanwhile, comparable validations and verifications have also been done in other independent laboratories worldwide.

The ISO 15216 was developed as a reference standard to which new methods can be compared. Improvements over time were expected, and some have accordingly been made, though not (yet) incorporated in the ISO standard. For example, the detection of viral target RNA in nucleic acid samples from matrices known to contain inhibitory substances can be much improved by an additional clean-up of the nucleic acid extracts. This procedure, which may be column based, can result in removal of inhibitory substances and can reduce the need for dilution of RNA extracts prior to amplification.

It is recognized that current validation protocols, as collected in the ISO 16140-series, are not fully applicable for detection of viruses in food as unlike bacteria, they cannot be enriched in culture (this is also applicable to foodborne parasites). Anticipating future availability of methods that might better discriminate infectivity status, in 2022, a new project group within ISO/TC34/SC9/WG3 (PG8) was formed, with the working title "Microbiology of the food chain — Method validation — Part 8: Protocol for the validation of alternative methods against a reference method for viruses and parasites".

Besides the ISO 15216-1 and -2, select countries have also developed national standards. Some of these are quite similar to the ISO method. Methods developed by the U.S. Food and Drug Administration (U.S. FDA, 2022) include protocols for matrix-dependent concentration, extraction, and detection of enteric viruses. These include methods for green onion and leafy greens (romaine lettuce and spinach); soft fruit (fresh and frozen blackberries, raspberries, strawberries, pomegranate arils, and mixed fruit); molluscan shellfish (oysters, mussels, and clams); and scallops and finfish (tuna).

The U.S. FDA virus extraction methods are based on the use of ultracentrifugation for contamination levels of viruses from all matrices. Subsequently the viral concentrates are extracted using silica binding (Boom *et al.*, 1990) with the inclusion of column-based kits for the removal of inhibitory substances. The RT-qPCR detection assays for norovirus and HAV target the ORF 1/2 junction and the 5' non-coding region, respectively. Included are controls for extraction efficiency and an internal amplification control. The method also includes a Control Exclusion Assay used to differentiate hepatitis A virus wild-type from laboratory control strains. The methods were validated in interlaboratory studies between state and federal laboratories within the United States of America, as detailed in the appendices (U.S. FDA, 2022). To date, a direct comparison study of performance characteristics between the ISO 15216 and U.S. FDA methods has not been published.

It can, however, be noted that there are differences in the prescribed elements of the ISO and FDA methods. All FDA Bacteriological Analytical Manual (BAM) Chapter 26 protocols (U.S. FDA, 2023) are strictly prescribed with respect to the brand names of kits, oligonucleotide primer and probes sequences, cycling programmes and detection platforms to be used. This was done to ensure the specificity and sensitivity of the methods. The intention is that any changes or improvements in technology that would warrant an official update to the BAM will be implemented as needed. The ISO 15216 method is likewise strict in the protocol used for virus extraction from food, but for nucleic acid extraction it only requests that a silica binding (Boom technology)-based method is used, leaving the user room for a choice of kits, or even addition of further RNA purification steps. This flexibility allows laboratories to adapt to availability of kits in a changing market or in different parts of the world. Primers and probes are also flexible inside defined regions of the viral genomes, which could be important when future developments of primer/probe designs lead to improvement of the method, such that there is no need for a revision of the ISO standard. To ensure harmonization in results, ISO 15216 advises optimization and/or checks by the user when diverting from the informative Annex in their choice for oligonucleotides, detection kits and platforms.

There are several methods for the detection of HEV in foods which have been published in the scientific literature (Cook *et al.*, 2022). Currently however, there is no national or international pu standard. To address this, in December 2021 ISO formed a working group (ISO TC34 / SC9 / WG31), which began the development of a standard for detection of HEV in meat, liver, and meat products, and liver and liver products, using RT-qPCR. The methods will be broadly based on previously developed procedures which have undergone method validation by interlaboratory ring trials (Althof *et al.*, 2019; Trojnar, 2020). It is anticipated that a method will be ready for validation by late 2024.

8.2. CHALLENGES IN DETECTION OF VIRUSES IN FOOD

Interpretation of RT-qPCR results. A major limitation when using RT-qPCR is that detection does not differentiate between infectious and non-infectious virus. This is because RT-qPCR detects a fragment of viral genomic material, which can be present in both infectious and non-infectious particles. In some instances, fragments of viral genomic material can persist in a matrix such as water, for example, even after virus inactivation (Seitz *et al.*, 2011). Protocol variations to aid in molecular-based infectivity discrimination have been investigated and reviewed (Knight *et al.*, 2013; Knight *et al.*, 2016). These can be considered proxies for infectivity, but none to date mediate unambiguous discrimination of virus infectivity status (see discussion below in **8.4**). It is unlikely that any nucleic acid amplification-based technique will ever be adapted to provide a failsafe infectivity assay, and rapid infectivity determination must await the development of a fundamentally different technology. In addition, the impact of some extraction methods on capsid integrity and infectivity does suggest that these methods may not completely preserve virus infectivity (Langlet *et al.*, 2018).

For a valid test result from both ISO and FDA-BAM methods, all quality assurance parameters (positive and negative controls) must produce the expected results, including meeting the specified performance criteria for amplification and process controls that are used to track the efficiency of virus contamination levels and potential for RT-qPCR amplification inhibition (see above). To call a test result positive, ISO and FDA-BAM methods also require demonstration of log-linear amplification curves in the test sample. For any microbiologically based detection method, care should be taken to assure that best practices are in place in the analytical laboratory: proper containment, attention to pipetting and reagent segregation, and well-trained personnel, among other considerations. Accreditation by a national accreditation body is an option to assure the quality of laboratory results. The current ISO 15216 standard uses external amplification control RNA standards (EACs or EC RNA known as external control RNA), which are comprised of artificial RNA sequences which pose a possible risk for cross-over contamination in the analyses. When the EAC is amplified, it can produce signals that are difficult to distinguish from those of wild-type virus targets (D'Agostino and Cook, 2018). This risk can be contained by choosing the appropriate use of concentrations and pipetting strategies. It is possible, however, to distinguish the two by restriction enzyme analysis and/or by nucleic acid sequencing of the amplified product, but this requires extensive post-analysis procedures, and many laboratories lack the equipment, time, or trained personnel to perform them. In addition, the use of multiple negative extraction controls between samples, for which the number is adjusted to the expected fraction of positive samples, can help rule out the potential for false positive test results.

At the time of writing this report, standard protocols to address this interpretive issue are not in place, although a revision of the ISO 22174:2005 (ISO, 2005) is being considered, where the use of target-identical controls, such as the PCR-positive control, may be given appropriate caveats and recommendations to avoid cross-contamination.

Sequencing. Neither the ISO 15216 nor the FDA-BAM method requires the use of nucleic acid sequencing as confirmation that an amplicon corresponds to viral nucleic acid arising from natural contamination. For samples with low levels of contamination, the Sanger method remains the current benchmark when sequencing is applied (Purpari *et al.*, 2019; Filipa-Silva *et al.*, 2021; Woods and Burkhardt, 2010). In addition, sequencing has been used in microbial source tracking and surveillance sampling studies, to link strains between infected individuals and contaminated foods, or to aid in determining country-of-origin for an implicated product. It is widely acknowledged that when RT-qPCR signals are weak, either due to low virus concentrations or residual matrix-associated inhibition, sequence analysis becomes more complicated and is often impossible (Cook, Williams and D'Agostino, 2019), although some report success (Ollivier *et al.*, 2022; Woods *et al.*, 2016). It is important to note that detection and characterization of viruses from contaminated food samples associated with disease outbreaks remains challenging for various reasons including heterogenous distribution of low concentrations of the contaminant, differences in RT-qPCR detection limits for both detection and typing, the presence of multiple strains in a single outbreak, and availability of implicated product, among other factors. Even when viral RNA can be detected, directly linking strains by comparison to clinical samples is not always possible.

Next generation sequencing (NGS) and whole genome sequencing (WGS) approaches have been developed as alternative methods for detection and characterization of enteric viruses, primarily applied to clinical samples (Mancini *et al.*, 2019; Strubbia *et al.*, 2019; Itarte *et al.*, 2021). NGS methods are advantageous as they allow higher sequencing depth, resulting in increased sensitivity for characterization of multiple strains. However, they usually require higher copy number of viral RNA than is present in many naturally contaminated foods and obtaining sufficient reads and skilled bioinformatician labour can be a challenge. Methods that have been used to increase the number of viral reads include metabarcoding, other amplicon- and capture-based methods (Desdouits *et al.*, 2020). While NGS methods are being widely used in bacteriology when sequencing the whole genome, WGS is not yet applicable to routine analysis for viral contamination in foods and environmental samples.

Digital PCR. Additional molecular based methods such as digital PCR/ digital-droplet PCR are frequently being used for virus detection in environmental and food matrices. Digital PCR / digital droplet PCR can allow for more sensitive detection and show decreased interference from inhibitors. There are also some disadvantages, the main one being cost, both of the consumables and equipment. Digital PCR was used for sensitive detection of norovirus in shellfish (Yang *et al.*, 2022; Plante *et al.*, 2021) and berries (Sun *et al.*, 2019). In the future, digital PCR may be used more frequently with the extraction methods described above.

International adoption of testing. The current virus concentration and detection methods are complex, as they comprise many steps and rely on highly trained personnel. Thus, they are designed for deployment in specialized laboratories; this may hinder their implementation in middle- to low-income countries, which will pose a significant challenge if food safety criteria are eventually developed for foodborne viruses. Moreover, reagents, especially the RT-qPCR kits and nucleic extraction, are expensive and often only regionally available.

8.3. IMPLEMENTATION OF METHODS AROUND THE WORLD

The Centre for Environment, Fisheries and Aquaculture Science (CEFAS, the United Kingdom of Great Britain and Northern Ireland) led the dissemination of the core procedures of ISO 15216-1 to European Union (EU) member laboratories prior to the publication of the standards. CEFAS was both convenor of the CEN/TAG4, which later became the ISO 15216 working group and organizer of proficiency testing schemes for the detection of human norovirus and HAV in bivalve molluscan shellfish. It served as the European Reference Laboratory for Shellfish until Brexit. Materials supplied for these proficiency scheme tests often included the positive controls (i.e. dsDNA for amplification and quantification described in the ISO 15216-1:2017). Upon implementation of proficiency testing, reports (CEFAS, 2023) suggested that an increasing number of participants started to use the ISO method both within and outside Europe, particularly for the detection of enteric viruses in oysters (Avant *et al.*, 2017). After Brexit, the European Union Reference Laboratory (EURL) for Foodborne Viruses (including bivalve molluscs shellfish), affiliated with the Swedish Food Agency in Uppsala (2018), took on the task of promoting implementation of the published ISO 15216-1 and -2 in the official National Reference Laboratories (NRLs) within the European Union. Their major role is to harmonize the detection and quantification of enteric viruses in food amongst EU member states.

During the JEMRA Expert Consultation, participants were queried as to which method(s) were being used routinely in their countries, and most countries use ISO 15216 standards. Further discussion amongst the panel members continued around the reasons(s) why countries or competent authorities perform monitoring of enteric viruses in various food commodities, and why others do not. The major reasons an authority would choose to perform monitoring for enteric viruses included collection of data for risk assessment and risk management, or to inform industries upon positive test results so they could improve their food safety plans. There has been much data gathered from various testing initiatives, but much of it is not publicly available and often not acted upon. Positive test results may appear as a notification tool, for example, in the EU Rapid Alert System for Food and Feed (RASFF) portal, or through the Directorate-General for Health and Food Safety (DG SANTE) when countries outside the European Union are part of an outbreak situation.

Authorities may choose not to perform monitoring of viruses for various reasons as well. One important reason is the absence of established microbiological criteria, in the current legislation, regarding official control for human norovirus or HAV contamination in food. Microbiological criteria for human norovirus in oysters have been discussed in some regions (see for instance the EFSA (European Food Safety Authority) opinion on human norovirus in oysters from 2012) (EFSA, 2012) but has not yet moved forward. In addition, virus testing of food is expensive, in comparison to testing for common bacterial pathogens, perhaps as much as tenfold higher in cost. Moreover, methods based on molecular testing do not discriminate between viral genomes associated with infectious particles versus inactivated/non-infectious particles, leading to a lack of consensus on the public health significance of positive test results, although the presence of human norovirus or hepatitis A virus genomes on foods may be evidence of fecal contamination. Concerns have been raised that batch testing is not likely to lead to enhanced food safety because of small sample size and non-uniform distribution of viruses in a lot or batch of food, leading to a low probability of detection, even in the presence of the contaminant (Dirks *et al.*, 2024; Jaykus *et al.*, 2023). However, this may highlight the significance of positive findings when product testing is conducted. Testing is not always a realistic option for foodborne viral outbreaks or for source attribution since meal remnants are often no longer available, and prolonged incubation periods (particularly for HAV and HEV) complicate food history recall. Also, although they have been successfully applied in some instances (Li *et al.*, 2023; McClure *et al.*, 2022), appropriate food extraction methods are not always available and/or have poor virus recovery efficiency.

8.4. INFECTIVITY ASSAYS AND PROXIES

The development of an effective *in vitro* propagation system for the non-culturable enteric viruses would be of immense benefit to food safety. Such a system could then be used (i) for propagation of viral stocks; (ii) to test the effectivity of viral inactivation treatments or food manufacturing processes (e.g. heat, UV, alcohols); (iii) to characterize environmental persistence; (iv) to evaluate whether or not food or environmental samples are contaminated with infectious virus; (v) to directly compare RT-qPCR to infectivity results; and/or (vi) to answer a myriad of basic scientific questions about these viruses and their infection/pathogenesis processes. Unfortunately, there is a long way to go before effective *in vitro* propagation methods are widely available. This section describes the current status.

Hepatitis A virus

The first use of the HM-175/18f strain in conjunction with secondary primate kidney cells to characterize parameters critical to food virology, in this case thermal inactivation, was reported by Perry and Mortimer in 1984 (Parry and Mortier, 1984). Into the 1990's, the cell culture method evolved and was used largely to aid in the evaluation of RT-PCR-based detection methods, particularly in bivalve molluscan shellfish (Jaykus *et al.*, 1995). The HM-175/18f strain and associated monkey kidney cell lines remain in use for research purposes. However, it is still not possible to routinely propagate infectious wild-type hepatitis A virus in the laboratory.

Human norovirus

Jones *et al.* (2014) were the first to report the propagation of human norovirus GII.4 (Sydney) in a human B lymphocyte cell line, a process that required either the addition of synthetic HBGA or the presence of enteric bacteria producing HBGAs. While providing evidence that human norovirus can infect immune cells and elucidating a potential co-factor promoting infection, the efficiency of replication for this method was low, and it has been difficult to reproduce. In 2016, Ettayebi *et al.* reported replication of human norovirus GII.4 variants in stem cell-derived human enteroids (HIEs). This method has been replicated by many others and has been widely used as a research tool since its first reporting (reviewed by Ettayebi *et al.*, 2021). HIE cultures, sometimes called "mini-guts," contain various cell types. While the highest replication efficiency, measured as an increase of detectable genome copies (GC), has been observed for the GII.4 strains (up to 4 $\log_{10}$ increase in GC), replication of other genotypes (e.g. GII.2, GII.3, GII.17, GI.1) has been achieved, albeit usually with less efficiency (Estes *et al.*, 2019).

Replication of some genotypes is influenced by the presence of bile. In general, replication is not only strain-dependent but also sample matrix-dependent. In other words, given the exact same strain, replication may occur with one fecal sample but not with another. This suggests that culture parameters need to be optimized by strain and matrix characteristics. The cultivation system has been reproduced in a number of laboratories (e.g. Alvarado *et al.*, 2018; Costantini *et al.*, 2018; Koromyslova *et al.*, 2019; Lindesmith *et al.*, 2019; Chan *et al.*, 2019).

While an exciting scientific development that may eventually lead to simpler cell lines and perhaps even a reliably cultivable strain like HM175/18f for HAV, the HIE system remains complicated, cumbersome, and costly (Estes *et al.*, 2019). Because no visible cytopathic effects are apparent, the system relies on RT-qPCR or RT-dPCR detection immediately after inoculation and 72 hours (or less) post-inoculation. Comparing the quantitative genome copies numbers between these two-time points leads to an estimation of viral replication, expressed as $\log_{10}$ GC increase. For the same clinical sample, GC increases can vary from assay to assay. In addition, the differentiated HIEs are sensitive to cytotoxicity, meaning that the sample matrix can hinder virus replication. For food and environmental samples, this is a real problem as these matrices may harbour such substances that can end up in viral extracts. Such extracts are required for liberation and contamination levels of viruses from these matrices, in order to reach the minimum number of viruses necessary to infect the HIEs. Finally, while the method might be used to confirm that a physical or chemical treatment effectively destroys virus infectivity, it is not quantitative as it cannot measure the degree of $\log_{10}$ inactivation achieved by the treatment. All these factors complicate the use of this method in food virology applications.

In 2019, Van Dycke *et al.* reported the use of zebrafish larvae (*Danio rerio*) to obtain significant quantities (2–3 $\log_{10}$ GC increase) of human norovirus GI and GII (including GII.4). Although zebrafish are not highly genetically related to humans, they have orthologous disease-related genes and a comparable innate immune system that includes B and T lymphocytes, macrophages, and neutrophils. Their high fertilization rate, rapid development and inexpensive maintenance make them a promising candidate for viral propagation. The infection protocol consists of inoculating an extremely small volume (3 nl) of fecal suspension of high viral load (10^9 GC/ml) into the yolk of larvae aged 3 days post-fertilization. After 2 days of incubation, the larvae are lysed and the viruses are observed in the intestines, hematopoietic tissues, in the liver and pancreas using labelled antibodies. The method has been reproduced by a few other laboratories internationally (Cuvry *et al.*, 2022; Kim *et al.*, 2022), and there is a recent report that replication can even occur in zebrafish embryos (Tan, Gong and Li, 2023). While it is an exciting

development, the method has low sensitivity, the inoculum volume is extremely small, and the starting virus concentration needed to initiate robust replication is very high. In addition, the method is not yet quantitative. These factors limit its utility for food virology.

Ghosh *et al.* (2022) reported that salivary gland cell lines support the replication of human norovirus. For a GII.4 Sydney 2012 strain the replication approximated those reported for the HIE cultures, with an approximate one-thousandfold increase in GC between 6 and 96 hours post inoculation (Ghosh *et al.*, 2022).

Nonetheless, proof-of-concept studies using both the HIE and zebrafish models have been reported for evaluation of physical and chemical treatments on human norovirus infectivity (Table 6). These studies can be considered confirmatory in nature as both methods cannot yet yield data on $\log_{10}$ reduction in virus infectivity.

TABLE 6 Applications of the human norovirus *in vitro* culture systems

CULTURE SYSTEM	INACTIVATION STRATEGIES	REFERENCES
Enteroid cells	Ethanol; surface disinfectant	Escudero-Abarca *et al.*, 2020
	Chlorine	Constantini *et al.*, 2018
	Green tea	Randazzo *et al.*, 2020
	Freshwater Clams	Hayashi *et al.*, 2022
	Heat	Ettayebi *et al.*, 2016; Wales *et al.*, 2024; Shaffer *et al.*, 2024
	High pressure processing	Falcó *et al.*, 2023; Wales *et al.*, 2024
	Estuarine water	Rexin, Rachmadi and Hewitt, 2024
Zebrafish	Heat	Tan *et al.*, 2021
	UV	Tan, Gong and Li, 2023
	Brown algae extracts	Tan *et al.*, 2021

Sources: See References.

Hepatitis E virus

Hepatitis E virus antigen production was demonstrated as early as 1987 in the human hepatoma cell line PLC/PRF/5 after inoculation with human stool samples from non-A, non-B hepatitis cases (Pillot *et al.*, 1987). In the ensuing years, reports of successful HEV replication in various cell lines were published, but replication efficiency was poor. More significant progress was experienced with the isolation

of HEV strains Kernow-C1/p6 (genotype 3a) and 47832c (genotype 3c) from chronically infected patients. These strains contain specific host or viral genomic insertions in the ORF1 hypervariable region that enhance their replication in cell culture (Shukla *et al.*, 2011; Johne *et al.*, 2014). In combination with specific cellular subclones (e.g. the human hepatoma cell line HepG2/C3A and the human lung cancer-derived cell line A549/D3), these and other HEV isolates were broadly used to investigate the virus' stability under different physico-chemical conditions, and in food or the environment (Table 7). However, replication in these cells lines is slow and does not result in cytopathic effects, meaning that techniques like immunofluorescence are necessary for identification of replication. Recently, several HEV strains have been isolated from pig and wild boar liver samples by culture in PLC/PRF/5 (human hepatocarcinoma cells) or A549/D3 (human lung adenocarcinoma cells) (Schilling-Loeffler *et al.*, 2021; Gremmel *et al.*, 2022; Stunnenberg *et al.*, 2023), but these systems remain laborious, time-consuming, and require high initial virus contamination levels, making them unsuitable for routine use at the current time.

TABLE 7 Applications of HEV cell culture systems

INACTIVATION STRATEGIES	HEV STRAIN	CELL LINE	REFERENCES
Chlorine	SAR-55	Caco-2/C25j	Girones *et al.*, 2014
Salt	47832c	A549/D3	Wolff *et al.*, 2020a
Disinfectants	Kernow-C1/p6	HepG2/C3A	Behrendt *et al.*, 2022
pH	47832c	A549/D3	Wolff *et al.*, 2020b
UV	Kernow-C1/p6	HepG2/C3A	Guerrero-Latorre *et al.*, 2016
Heat	47832c	A549/D3	Johne *et al.*, 2016
Heating in meat matrix	83-2, 121-12	PLC/PRF/5	Imagawa *et al.*, 2018
	14-16753, 14-22707	A549/D3	Stunnenberg *et al.*, 2023
Drying on surfaces	47832c	A549/D3	Wolff, Günther and Johne, 2022
High hydrostatic pressure	47832c	A549/D3	Johne *et al.*, 2021
	47832c	A549/D3	Nasheri *et al.*, 2020

Sources: See References.

Cultivable animal viruses as human norovirus surrogates

In the absence of methods to propagate human norovirus, cultivable surrogate viruses are often used in lab-based studies, as proxies, to characterize virus behaviours and to evaluate the efficacy of prevention and control strategies. Prior to 2007, the respiratory feline calicivirus (FCV) was widely used for this purpose, but there were concerns that it was more sensitive than human norovirus to many treatments. Murine norovirus (MNV), which causes neurological illness in mice, has a capsid structure, genomic organization, and replication cycle very similar to human norovirus (Wobus, Thackray and Virgin, 2006). It emerged as an alternative surrogate, first applied in food virology applications by Cannon *et al.* (2006) and used in comparative disinfection studies two years later (Belliot *et al.*, 2008). It continues to be widely used to study various aspects of human norovirus replication as well as used as a model for the behaviour of human norovirus exposed to various physical and chemical stressors. Tulane virus (TuV), recovered from the fecal material of a captive macaque (Farkas *et al.*, 2008) is a calicivirus that can be readily propagated in a monkey kidney cell line, with the appearance of typical cytopathic effects. It was used as a cultivable human norovirus surrogate as early as 2012–2013 (DiCaprio *et al.*, 2012; Tian *et al.*, 2013). In perhaps the most comprehensive comparative study among the candidate human norovirus surrogates (Cromeans *et al.*, 2014), FCV was found to be relatively more sensitive to acidic pH, chlorine, and high pressure, while MNV was more sensitive to alcohols and high pressure. Based on the totality of the data, the authors concluded that TuV and MNV were the most resistant of the relevant cultivable surrogates tested and should be considered the best candidates to serve as proxies for human norovirus. Over the last decade, researchers have increasingly used TuV as a reliable human norovirus surrogate (Hirneisen and Kniel, 2013) although this virus is not yet available commercially. To be cautious, the choice of appropriate surrogate is dependent on the nature of the study and the characteristics of the virus, including genetic similarities, binding properties, and ease of propagation, among others (Kniel, 2014).

Proxies for virus infectivity

RT-qPCR detects nucleic acid from both infectious and non-infectious virus. Therefore, scientists have also been investigating alternative molecular methods as a proxy for viral infectivity, with most of that work focusing on human norovirus (Table 8).

TABLE 8 Examples of proxies for virus infectivity RNA extraction pre-treatments

PRE-TREATMENT STRATEGIES	FOODBORNE VIRUS TARGETED	REFERENCES
PMA, EMA and PMAxx	norovirus, hepatitis A virus, hepatitis E virus	Randazzo *et al.*, 2016; Chen *et al.*, 2020; Sánchez, Elizaquível and Aznar, 2012; Randazzo *et al.*, 2018
$PtCl_4$	norovirus, hepatitis A virus, hepatitis E virus	Fraisse *et al.* 2018; Randazzo *et al.*, 2018
RNase	feline calicivirus, hepatitis A virus, poliovirus, norovirus	Nuanualsuwan and Cliver, 2002; Lamhoujeb *et al.* 2008

Sources: See References.

Viability pre-treatments, intended to remove or make viral RNA unavailable for subsequent amplification, are the most often investigated approaches to infectivity proxies. The two most commonly reported methods are RNase pre-treatment and nucleic acid intercalating dyes. These methods effectively degrade free RNA, or otherwise prevent unencapsidated RNA from being amplified by RT-qPCR. Theoretically, the only RNA that will be amplified should be that associated with full-integrity capsids, i.e. infectious virus.

RNase degrades single-stranded RNA and works best on free RNA, although if the RNA is associated with capsid protein or damaged capsids, it is possible to include a proteinase pre-treatment which theoretically allows RNase access to the genome. Most studies on this method have not included proteinase. They do not penetrate intact virus capsids, but they can penetrate destroyed or damaged capsids and covalently intercalate the RNA after being activated by strong visible light. This binding to the genome interferes with RT-qPCR amplification (Randazzo *et al.*, 2016). Platinum compounds (e.g. platinum [IV] chloride [PtCl4]) bind to nucleic acids but also to specific amino acid residues (Rosenberg *et al.*, 1965; Serrano *et al.*, 2011; Soejima and Iwatsuki, 2016), and these compounds are a particularly appealing method because it is less sensitive to light and inexpensive (Soejima *et al.*, 2016). Recent applications to foods are shown in Table 8. RNase pre-treatment and nucleic acid intercalating dyes are both applied prior to RNA extraction, so upstream viral extraction as described in the ISO 15216 may remain unchanged. However, it should be noted that for bivalve molluscan shellfish, the proteinase K treatment to liberate the viruses from the tissue was shown to effect viral integrity (Langlet *et al.*, 2018).

Other approaches to estimate the infectivity of foodborne viruses aim to obtain information on (i) genome completeness (e.g. by long-range PCR), or (ii) the binding capacity of the viruses to host cells or target-specific ligands (e.g. HBGAs, porcine gastric mucin binding assays, nucleic acid aptamers, antibodies, and peptides). These are reviewed elsewhere (Dirks *et al.*, 2024).

Suffice it to say that despite substantial efforts in methods development, none of the current candidates comes to the forefront. Complications include but are not limited to an inability to reliably replicate the method; sensitivity; complexity and expense; applicability to all virus strains and/or matrices; proof of true association between detection and infectivity; and consistent performance over a myriad of inactivation methods, both physical and chemical. While each of the proxy assays provide partial efficacy, combinations or as-yet identified alternatives may be required to obtain better correlation between molecular-based detection and virus infectivity.

8.5. INDICATOR ORGANISMS

Microbiological indicators are organisms whose presence and/or concentration(s) have an association with the presence of one or more pathogens in a food or the environment. They can be bacteria or viruses, occasionally a metabolic product thereof. The ideal indicator should be non-pathogenic, easy to detect/quantify, have similar stability and morphology to its associated pathogen(s), preferably similar survival rates in the environment, and be typically present in greater number than the pathogen(s). The most commonly used fecal indicator bacteria (FIB) are fecal coliforms and generic *Escherichia coli*, both of which are associated with the fecal material of warm-blooded animals. Current FIBs serve as a predictor of fecal contamination and have been successful in preventing bacterial gastrointestinal infections, particularly infections like shigellosis. In most countries, FIB are used to determine or assure drinking water microbiological safety, adequate treatment of water, sanitary conditions in food production, and microbiological water quality of recreational and shellfish waters (National Research Council, 2004; Worley Morse *et al.*, 2019; FAO and WHO, 2023). While *E. coli* and fecal coliforms are the most widely used FIB, in practice they are believed to have limited predictive value for viral enteric pathogen contamination (Pina *et al.*, 1998; Goyal, 2006).

Because of the relatively poor predictive value of *E. coli* and fecal coliforms, alternative indicators for enteric viral pathogens have been proposed (summarized in Table 9). Due to their capsid morphology, environmental abundance and stability, and ease of propagation and detection, male specific coliphages (MSC) have long been studied as an alternative indicator for human enteric virus contamination (Goblick *et al.*, 2011; Doré and Lees, 1995; Pouillot *et al.*, 2015; Pouillot *et al.*, 2022). The use of MSC to examine shellfish meats for sanitary shoreline surveys and emergency closure events is outlined in the U.S. National Shellfish Sanitation Program Guide for the Control of Molluscan Shellfish, 2019 Revision (U.S. FDA, 2019). Male specific coliphages, and other indicators, have also been used in shellfish surveillance and

sampling studies to investigate their ability to predict enteric virus contamination (DePaola *et al.*, 2010; da Silva *et al.*, 2023; Lowther, Henshilwood and Lees, 2008). In these studies, there was some correlation between the presence of MSC when enteric viruses were detected. However, in instances where MSC were analyzed in shellfish-associated viral outbreaks, for which the source of contamination may not have been well-documented, the MSC did not appreciably predict the presence of viral pathogens (Woods *et al.*, 2016). Other than for use in molluscan shellfish, there is no large-scale adoption of this viral indicator to index for potential enteric virus contamination of other food commodities.

Most of the proposed indicators that have been studied over the last decade have been investigated in water, wastewater, and sewage treatment. While none appear as prominent candidates, the DNA cross assembly phage (crAssphage) has been widely studied as a potential indicator of human fecal contamination in water and wastewater (Sabar, Honda and Haramoto, 2022; Park *et al.*, 2020). Its abundance in human feces and its environmental stability make crAssphage a potentially effective candidate for this purpose, yet additional studies are necessary to determine its feasibility for use as an indicator of enteric virus contamination of food (Stachler and Bibby 2014). Unfortunately, there are no viability (plaque) assays available for the detection of crAssphage.

Adenoviruses are DNA viruses that were proposed as viral indicators in the early 1990s (Pina *et al.*, 1998). They are found in wastewater at concentrations up to 10^8 genomic copies/L, and some strains can be propagated and infectious units determined (Girones *et al.*, 2014). Because some of these DNA viruses can be pathogenic to humans, they would not be considered an ideal viral indicator. In the late 2000s, the plant virus pepper mild mottle virus (PMMoV) emerged as a potential indicator, as the genetic material of PMMoV can be found in the stool of humans and in wastewater (Colson *et al.*, 2010). This virus has been used as a process indicator in wastewater treatment plant studies, and like crAssphage, has been suggested as an indicator of fecal contamination (Rosario *et al.*, 2009). One study suggested that PMMoV might produce a specific immune response and clinical symptoms in humans (Colson *et al.*, 2010). Somatic coliphages, *Bacteroides*, and bacteriophage infecting *Bacteroides* are additional candidate indicators of fecal contamination whose primary uses to date have been to monitor water quality and to track microbial sources (Table 9).

Ideally, an indicator will predict the presence of the pathogen(s) with some relationship to the degree of public health risk. While the indicators described in Table 9 continue to be studied, none of these candidates yet meet the criteria for their widespread use as indicators for enteric virus contamination of food.

TABLE 9 Summary of microbiological indicator candidates for use in food and environmental virology

INDICATOR ORGANISM	PROPERTIES	CURRENT USES	CHALLENGES	REFERENCES
E. coli	Gram negative bacteria, mammalian source (humans and animals)	Used by most regulatory bodies as an indicator of fecal contamination	Can replicate in the environment, cannot distinguish between animal and human contamination, does not indicate presence of enteric viruses	Winterbourn *et al.*, 2016; Phanuwan *et al.*, 2006
F+ coliphages (male specific coliphages)	Primarily ssRNA virus, mammalian source (humans and animals), can propagate and enumerate with plaque assay *E. coli* F-amp host bacteria. Also detected by RT-qPCR (genogroup). Typically present in greater number than viral pathogens	Analysis of shellfish meats and microbial water quality	Not consistently present in molluscan shellfish associated with enteric virus outbreaks. Not all genogroups are easily propagated	Worley-Morse *et al.*, 2019; Woods *et al.*, 2016; Pouillot *et al.*, 2015; Pouillot *et al.*, 2022
Somatic coliphages	dsDNA virus, mammalian source (humans and animals), can propagate and enumerate with plaque assay *E. coli* CN-13 host bacteria	Microbial water quality	Can propagate in the environment	Silverman *et al.*, 2013; Jofre, 2009
Cross-assembly phage (crAssphage)	dsDNA virus, typically present in greater number than viral pathogens	Primarily human fecal origin, research base only as human fecal indicator	Cannot propagate *in vitro* or determine viability. Detected by qPCR	Sabar, Honda and Haramoto, 2022; Park *et al.*, 2020; Gyawali *et al.*, 2021; Malla *et al.*, 2019
Pepper mild mottle virus (PMMoV)	ssRNA virus, plant pathogen, typically present in greater number than viral pathogens	Research base as viral indicator, microbial water quality	Not a human-specific indicator	Shrestha *et al.*, 2018; Yasui *et al.*, 2021; Rosario *et al.*, 2009; Gyawali *et al.*, 2019; Malla *et al.*, 2019
Adenovirus	dsDNA virus, can propagate some stains, human and animal strains	Research base as viral indicator	Human pathogen	Rames *et al.*, 2016
Bacteroides and bacteriophage infecting *Bacteroides*	Gram negative bacteria, anaerobic, DNA genetic material	Research based as human fecal indicator, microbial source tracking	Extensive methods to propagate; usually use qPCR for detection	Silverman *et al.*, 2013; Sauer *et al.*, 2011

Sources: See References.

8.6. CONCLUSIONS

Since the 2007 FAO/WHO expert consultation (FAO and WHO, 2008), standardized detection and quantification methods for enteric viruses in food have been developed, validated and published. The ISO 15216 was first published as a technical specification in 2013 and more recently, as a validated standard in two parts, quantitative and qualitative. Methods are described for detection of human norovirus GI and GII, and hepatitis A virus, in the following matrices/sample types: bivalve molluscan shellfish; soft fruit; leaf, stem, and bulb vegetables; bottled water; and food (preparation) surfaces. The ISO 15216 standard is widely used by competent authorities in the European Union and elsewhere in the world, including several countries which use the ISO methods with minor modifications. It is also the method of choice for commercial testing laboratories (personal communication). The U.S. FDA interlaboratory validated methods for the extraction and detection of hepatitis A virus from green onion were published as Bacteriological Analytical Manual (BAM) Chapter 26B (U.S. FDA, 2023) and made publicly available in 2013. The additional matrices and the norovirus RT-qPCR assay were officially released in 2022 and cover the following matrices: green onion and leafy greens; soft fruit; molluscan shellfish; and scallops and finfish, also for both hepatitis A virus and GI/GII human norovirus. The FDA-BAM method is used exclusively in the United States of America. Currently, there are no standardized methods available for detecting enteric viruses in other matrices or for detecting other viruses. However, a standardized method for HEV is currently in development.

While the availability of standardized, validated methods is a huge step forward, their widespread use is limited by factors such as high cost and methodological complexity, and applicability to limited sample matrices, although the ISO 15216 and FDA BAM Chapter 26 include methods for produce and shellfish, commodities implicated in most foodborne viral outbreaks. These methods have been used in the last decade for outbreak investigation and surveillance studies, but other uses are still limited.

Most recent efforts at cultivation have focused on human norovirus, and several cultivation methods have been reported in the literature over the last seven years. These methods were based on virus propagation in stem cell-derived human enteroids, B-cells, zebrafish larvae and embryos, and saliva gland cells. While potentially useful research tools (to study virus replication, for example), they lack key features necessary for their practical application to the detection of naturally contaminated enteric viruses on foods, or to study the efficacy of prevention and control methods. Efforts have gone into establishing cultivation methods for HEV with some promising results.

In the absence of virus cultivation methods, microbiological indicators continue to be an important tool in managing potential enteric virus contamination of foods. While the fecal indicator bacteria *E. coli* and fecal coliforms are still widely used, in practice they have limited predictive value for enteric virus contamination. Since the 2007 FAO/WHO expert consultation, some novel indicators have been identified and investigated, mostly for use in managing water and wastewater. Major candidates have included DNA cross-assembly phage (crAssphage), human adenovirus, pepper mild mottle virus (PMMoV), and various bacteriophages. Further studies are in order, to determine or find the ideal candidate for a microbiological indicator.

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9

Conclusions and recommendations

Foodborne viruses and foods of highest public health concern

During the expert meeting, human norovirus was identified as the leading cause of viral foodborne illness, followed by hepatitis A and hepatitis E viruses. Hepatitis A virus and hepatitis E virus were ranked equally but higher compared to norovirus in terms of clinical severity. When considering both frequency and severity, the ranking for these viruses fell into three groups as follows:

1. norovirus
2. hepatitis A virus and hepatitis E virus ranked in order
3. rotavirus, sapovirus, enterovirus, astrovirus, and enteric adenovirus ranked in order

The Expert Committee considered commodities from a global perspective and identified the virus-commodity pairs of highest global public health burden associated with specific viruses (Table 1).

The Expert Committee acknowledged the lack of sufficient data to conduct a ranking of foods that may be contaminated by astrovirus, sapovirus, enterovirus, enteric adenovirus, and rotavirus. To address the collective need for more data, countries should enhance investigation of foodborne illness and/or relevant foods for these viruses. Ranking of virus commodity pairs on a global level is challenging; this is partially due to regional differences in foods attributed to human viral foodborne illness; virus circulation among persons; regional variations in food production, consumption and preparation patterns; and to immune and nutritional status.

Viral foodborne disease has a substantial impact in terms of morbidity and mortality. Globally, the lack of surveillance data, the potential for asymptomatic shedding, and sparse reporting of foodborne cases pose major challenges to prevention and control strategies.

Each year, norovirus is estimated to cause 125 million cases of foodborne illness and 35 000 deaths globally. Norovirus is highly infectious, and outbreaks have been linked to foods with low levels of contamination. Viral contamination can occur over the whole food chain. Severe outcomes including hospitalization and death mainly affect children less than 5 years of age, the elderly, and immunosuppressed individuals who may shed the virus for extended periods of time. Most norovirus genotypes and variants are detected globally while regional differences exist.

Hepatitis A virus is estimated to cause 14 million cases of foodborne illness and 28 000 deaths globally each year and is a reportable disease in some countries. There are distinct geographical distributions of HAV genotypes, so even partial genomic sequencing can aid in source identification during outbreak investigations. There are also significant regional differences in the proportion of hepatitis A cases that are attributed to food due to endemic prevalence and vaccine utilization. International trade of foods plays an important role in transmission to susceptible populations. Wider compliance with international standards, e.g. good agricultural and hygiene practices, is likely to reduce global transmission.

Hepatitis E virus is unique among the foodborne viruses in that it is a zoonotic pathogen with many asymptomatic animal reservoirs, most notably swine. While there is no global estimation of cases attributed to food, countries that have investigated further have found that their prior estimates are too low by one order of magnitude or more. Genotypes 3 and 4 originating from infected animals are major sources for foodborne cases of hepatitis E, a trend that has been increasing in recent years in some countries. These genotypes cause acute hepatitis which can be severe in individuals with underlying health conditions. They cause chronic hepatitis leading to cirrhosis and liver damage in people with immunocompromised conditions and are associated with a wide range of neurological sequalae. Undercooked pig products including liver or raw sausage containing liver or blood, as well as liver pâté, are the main foods contaminated by hepatitis E virus.

Analytical methods and indicators for foodborne viruses

Since the 2008 JEMRA report on viruses in foods, international and national standard methods have been developed and validated for detection and quantification of human norovirus and hepatitis A virus in foods. The methods have been implemented in various countries. The International Organization for Standardization (ISO) methods ISO-15216-1:2017 and ISO-15216-2:2019 are widely used for the detection of norovirus and hepatitis A virus in various commodities and are likely to become benchmarks for validation of new methods. Matrices included in these ISO methods are, for example, leafy greens, soft fruits, and shellfish. ISO methods for hepatitis E virus detection in meats and meat products are in development. National methods, aside from ISO methods, have been validated and are being used by some laboratories. Current standardized methods are based on detection of viral nucleic acid, which does not necessarily indicate virus infectivity. The utility of the methods can be limited by several other factors (e.g. the complexity of the food composition, low levels of contamination). Despite the methodological advancements, there remain challenges in the use of the current standardized methods, most notably ensuring accurate interpretation; application to other viruses and/or matrices; integration of sequencing technologies; and implementation in low resource countries. Sharing of laboratory and epidemiological data, nationally, regionally, and internationally can improve the understanding and control of foodborne viruses.

Most recent efforts at cultivation have focused on human norovirus, and four cultivation methods have been reported in the literature over the last seven years (i.e., B-cells; human enteroids; zebrafish larvae and embryos; saliva gland cells). While potentially useful research tools (to study virus replication, for example), they lack key features necessary for their practical application to the detection of enteric viruses in naturally contaminated foods, or to study efficacy of prevention and control methods. Efforts have gone into establishing cultivation methods for HEV with some promising results.

A variety of indicators for viral contamination have been investigated, and major candidates have included DNA cross-assembly phage (crAssphage), human adenovirus, pepper mild mottle virus (PMMoV), various bacteriophages, and *Bacteroides* spp. Up to this point, these have been mostly studied in environmental waters and shellfish, with variable utility. Additional research is needed to determine if there is an appropriate viral indicator for use in other commodities associated with foodborne virus contamination.

The above emphasizes the importance of a global surveillance programme across the food system that will generate data to provide critical missing assistance with the management and control of viral pathogen concerns in foods, reducing the danger of consuming contaminated foods.

10

Needs assessment and data gaps

The meeting identified numerous data gaps, which if filled would greatly contribute to the level of knowledge of foodborne viruses.

- There is a need for infectivity assays for wild-type enteric viruses, relative to detection in foods and their environments. Despite the existence of multiple experimental approaches, there is still no definitive means to differentiate infectious from non-infectious viruses using molecular amplification. Infectivity assays will also aid in the ability to evaluate the efficacy of candidate virus inactivation methods.
- The Expert Committee recommends that member countries consider capacity building to support training and adoption of these methods for detecting viruses in foods and the environment. This has the potential to enhance knowledge on food attribution, support risk analysis, and reduce the burden of viral foodborne disease worldwide.
- Better characterization of the prevalence of viral contamination in various food commodities, separate from water, is needed to fully understand viral attribution, and this is particularly important in low- and middle-income countries.
- Challenges associated with increased use of molecular detection and the need for cautious interpretation must continue to be evaluated. Molecular detection does not necessarily indicate the presence of infectious viruses but merely the presence of (fragments of) the viral genome.
- Climate change is impacting human lives and human health in many ways. This includes resources essential to health, like clean air, safe drinking water, and a nutritious and safe food supply. The various areas in which climate change may impact foodborne virus transmission should be further considered in the context of epidemiological attribution and public health.

Annexes

Annex 1

Scoping review

ANNEX 1.1. THE KEYWORDS USED IN THE LITERATURE SURVEY FOR OUTBREAK, MONITORING AND SURVEILLANCE DATA

TABLE A1 The keywords

virus or norovirus or NoV or "hepatitis A" or HAV or "hepatitis E" or HEV or rotavirus or HRV or coronavirus or CoV or "Nipah virus" or HPAI-H5N1 or "Influenza A" or "Enteric Adenovirus" or sapovirus or astrovirus or "aichi virus" or enterovirus or poliovirus or parechovirus or "tick-borne encephalitis virus"	AND
food or foodborne or meat or beef or pork or lamb or poultry or chicken or turkey or game or dairy or milk or cheese or yogurt or cream or "milk powder" or "cream powder" egg or fat or butter or fish or shellfish or crustaceans or echinoderms or molluscs or bivalve or shrimp or crab or oyster or mussel or oil or sugar or cereal or grain or bean or flour or pasta or noodle or bread or soybean or nut or seed or honey or syrup or "ice cream" or confectionery or cocoa or candy or chocolate or sweet or dessert or gum or bakery or cracker or bagel or pita or muffin or cake or cookie or pie or doughnut or roll or scone or produce or vegetable or fungi or mushroom or herb or root or bulb or tuber or seed or seeded or solanaceous or vine or legume or pulse or sprouts or stem or leafy or flower or seaweed or salad or fruit or berry or melon or stone or pome or jam or jelly or marmalade or vinegar or spice or soup or salad or seasoning or condiment or sauce or dip or vinegar or mustard or beverage or "portable water" or "drinking water" or juice or "edible ice" or sherbet or sorbet or nectar or coffee or lettuce or coleslaw or "leafy green" or raspberry or strawberry	AND
outbreak or monitor or monitoring or surveillance	AND
COVID or SARS or "yellow fever" or "chicken pox" or "stem cell" or "HIV"	NOT

ANNEX 1.2. THE QUESTIONS FOR THE TWO-STEP RELEVANCE SCREENING AND CONFIRMATION USED IN THE LITERATURE SURVEY FOR OUTBREAK, MONITORING AND SURVEILLANCE DATA

A1.2.1 Relevance screening

TABLE A2 Relevance screening

QUESTION	OPTION
Does this citation describe research on foodborne virus outbreak or monitoring or surveillance?	• Yes, primary research • Yes, systematic review/meta-analysis • Yes, risk assessment, risk profile, or other risk-based tool (e.g. cost-benefit analysis) • No

Definition

- **Primary research** is collection of new data in a single study.
- **Systematic review** is a structured review of a clearly defined question with a transparent search strategy, relevance screening process, data extraction, risk-of-bias assessment and synthesis of results.
- **Meta-analysis** is a statistical technique that can be used on data collected in a systematic review.
- **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.

A1.2.2 Relevance confirmation

TABLE A3 Relevance confirmation

QUESTION	OPTION
1) Type of study	• outbreak • others (monitoring, prevalence, etc.)
2) Which viruses	• norovirus • hepatitis A • hepatitis E • rotavirus • coronavirus • others
3) Country	
4) Food	
5) Contamination source	• Primary • Processing • Retail • Consumption
6) Year (of outbreak or monitoring or surveillance)	
7) Age of the patient	
8) Number of cases	
9) Number of hospitalized	
10) Number of deaths	
11) Other type of data	

Annex 2

Methods for database and literature survey for global burden and sources of disease data

The review was focused on the collecting of source attribution studies conducted globally in the period of 2000–2023 and attributing the human cases of foodborne virus diseases to the general types of transmission such as foodborne and environmental transmission including animal contact, transmission from person to person, and specific foods.

SEARCH STRATEGY AND STUDY SELECTION

The initial literature search was done in Pubmed Medline database. An exploratory search string was created and piloted in Pubmed, then it was updated and the final search string, which covered the most relevant studies of the foci of this review, was chosen. To narrow down the results of the search to the studies addressing the source attribution of human foodborne diseases, the terms "human" OR "clinical" OR "disease" was added. Also, the names of the viruses of interest were used as the search terms (#2). First, the period 2010–2023 for the literature search was used, but then, due to the lack of the studies found, it was widened up to 2000–2023 to cover more available studies. Table A4 summarizes the search terms and the number of the retrieved records. No language restrictions were applied; however, the searches were only carried out in English. Then, the literature search proceeded in Google scholar with the terms "attribution, virus, food".

Assessment of the identified articles was carried out using eligibility criteria by screening the titles, abstracts, and, where necessary, quick estimation of full texts.

TABLE A4 Search terms used in PubMed database to retrieve articles and number of records searched for screening

SEARCH TERMS	YEAR FILTER APPLIED	NUMBER OF RECORDS
(Source attribution OR attribution) AND Foodborne virus	2010–2023	90
(Source attribution OR attribution) AND Foodborne norovirus	2010–2023	62
(Source attribution OR attribution) AND Foodborne hepatitis	2010–2023	26
Attribution AND Foodborne AND #1 AND #2	2000–2023	100
Source AND Food AND #1 AND #2	2000–2023	1 270

Notes: #1 – Human OR clinical OR disease; #2 – Norovirus OR Rotavirus OR Adenovirus OR Sapovirus OR Astrovirus OR Nipah virus OR Aichi virus OR Hepatitis OR Enterovirus OR HPAI virus H5N1 OR SARS

ELIGIBILITY CRITERIA

In the initial formal search, only i) the source attribution studies, ii) conducted on datasets collected between 2000–2023, and iii) attributed human cases of foodborne diseases to the sources of infection, were selected.

This means that the studies that report the source attribution estimates (e.g. the proportion of attributed cases, the proportion of attributed outbreaks, and the percentages of foodborne illnesses or human cases attributed to the source, or the estimates revealed a statistically significant association with illness and consumption of certain food [OR, RR]) were selected. No language criteria were applied.

The foodborne virus list was defined as provided by the JEMRA secretariat. It included: norovirus, Group A rotavirus, Group B, C rotavirus, Enteric Adenovirus, Sapovirus, Astrovirus, Aichi virus, hepatitis A virus, hepatitis E virus, enterovirus, Nipah virus, HPAI virus H5N1, and SARS Coronavirus.

DATA EXTRACTION

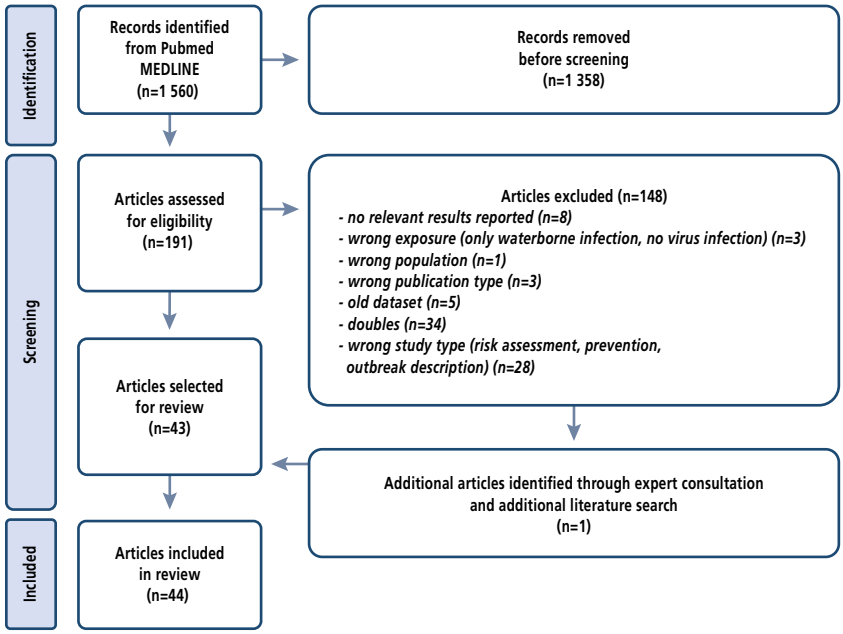
The data were extracted into an MS Excel table. The following variables were extracted: bibliographic reference of the study, DOI, title, year of publication, source attribution approach, country, or region where data samples were collected, period of data collection, virus name, data sources, the analytical method including

uncertainties assessment, the type of source attribution estimates, and the values of source attribution estimates (e.g. source attribution data). During the data extraction, the hazards labelled as "Norwalk-like virus" were changed to "Norovirus".

RESULTS

Forty-four studies reporting data on the sources of foodborne virus published globally between 2000 and 2023 (Figure A1) were identified. From the initial 1 560 hits identified and the removal of 1 358 articles that were not relevant during initial screening, 148 studies that did not match the inclusion criteria were excluded.

FIGURE A1 The flow chart of the literature search



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1 FAO and WHO. 2002. Risk assessments of *Salmonella* in eggs and broiler chickens: interpretative summary.

2 FAO and WHO. 2022. Risk assessments of *Salmonella* in eggs and broiler chickens.

3 FAO and WHO. 2003. Hazard characterization for pathogens in food and water: guidelines.

4 FAO and WHO. 2004. Risk assessment of *Listeria monocytogenes* in ready-to-eat foods: interpretative summary.

5 FAO and WHO. 2004. Risk assessment of *Listeria monocytogenes* in ready-to-eat foods: technical report.

6 FAO and WHO. 2004. *Enterobacter sakazakii* and other microorganisms in powdered infant formula: meeting report.

7 FAO and WHO. 2008. Exposure assessment of microbiological hazards in food: guidelines.

8 FAO and WHO. 2005. Risk assessment of *Vibrio vulnificus* in raw oysters: interpretative summary and technical report.

9 FAO and WHO. 2005. Risk assessment of choleragenic *Vibrio cholerae* O1 and O139 in warm-water shrimp in international trade: interpretative summary and technical report.

10 FAO and WHO. 2006. *Enterobacter sakazakii* and *Salmonella* in powdered infant formula: meeting report.

11 FAO and WHO. 2008. Risk assessment of *Campylobacter* spp. in broiler chickens: interpretative summary.

12 FAO and WHO. 2008. Risk assessment of *Campylobacter* spp. in broiler chickens: technical report.

13 FAO and WHO. 2008. Viruses in food: scientific advice to support risk management activities: meeting report.

14 FAO and WHO. 2008. Microbiological hazards in fresh leafy vegetables and herbs: meeting report.

15 FAO and WHO. 2008. *Enterobacter sakazakii* (*Cronobacter* spp.) in powdered follow-up formula: meeting report.

16 FAO and WHO. 2011. Risk assessment of *Vibrio parahaemolyticus* in seafood: interpretative summary and technical report.

17 FAO and WHO. 2009. Risk characterization of microbiological hazards in food: guidelines.

18 FAO and WHO. 2010. Enterohaemorragic *Escherichia coli* in raw beef and beef products: approaches for the provision of scientific advice, meeting report.

19 FAO and WHO. 2009. *Salmonella* and *Campylobacter* in chicken meat: meeting report.

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23 FAO and WHO. 2014. Multicriteria-based ranking for risk management of foodborne parasites.

24 FAO and WHO. 2016. Statistical aspects of microbiological criteria related to foods: a risk managers guide.

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26 FAO and WHO. 2022. Ranking of low moisture foods in support of microbiological risk management: meeting report and systematic review.

27 FAO and WHO. 2022. Microbiological hazards in spices and dried aromatic herbs: meeting report.

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29 FAO and WHO. 2021. Microbial safety of lipid based ready-to-use foods for the management of moderate acute and severe acute malnutrition: second report.

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31 FAO and WHO. 2018. Shiga toxin-producing *Escherichia coli* (STEC) and food: attribution, characterization, and monitoring.

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38 FAO and WHO. 2022. *Listeria monocytogenes* in ready-to-eat (RTE) foods: attribution, characterization and monitoring, meeting report.

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45 FAO and WHO. 2023. Measures for the control of *Salmonella* spp. in poultry meat, meeting report.

46 FAO and WHO. 2024. Measures for the control of *Campylobacter* spp. in chicken meat, meeting report.

47 FAO and WHO. Risk assessment of *Listeria monocytogenes* in foods - Part 1: Formal models, meeting report, in progress

48 FAO and WHO. Risk assessment of *Listeria monocytogenes* in foods - Part 2: Risk assessment, meeting report, in progress

49 FAO and WHO. 2024. Risk assessment of viruses in foods - Part 1: Food attribution, analytical methods and indicators, meeting report.

Microbiological Risk Assessment Series

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.

The Expert Committee: 1) reviewed the literature and available surveillance databases, and participated in an expert knowledge elicitation, which ranked foodborne viruses according to frequency and severity; 2) ranked the relevant food commodities of highest public health concern; 3) discussed methods for virus testing performed for outbreak investigation and product testing as part of surveillance and monitoring strategies; and 4) reviewed current and potential indicators for viral contamination.

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