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Viruses

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Abstract :

some diseases now known to be viral were recorded as foodborne before the nature of viruses was understood. In particular, outbreaks of poliomyelitis associated with drinking raw milk were recorded before the poliomyelitis viruses (polioviruses) had been isolated. Polioviruses only infect humans and other primates and are shed in feces. The problem of milk transmission of polioviruses, therefore, was a clear example of food handler–associated transmission, which was solved by improved sanitation and almost universal pasteurization of milk before vaccines became available and eradicated poliomyelitis in the developed world.

Keywords : Gastroenteritis viruses, Hepatitis A virus, Hepatitis E virus, Noroviruses, Viruses

s0010 # 14.1 INTRODUCTION

s0015 ## 14.1.1 HISTORY OF FOOD VIROLOGY

p0010 Some diseases now known to be viral were recorded as foodborne before the nature of viruses was understood. In particular, outbreaks of poliomyelitis associated with drinking raw milk were recorded before the poliomyelitis viruses (polioviruses) had been isolated. Polioviruses only infect humans and other primates and are shed in feces. The problem of milk transmission of polioviruses, therefore, was a clear example of food handler–associated transmission, which was solved by improved sanitation and almost universal pasteurization of milk before vaccines became available and eradicated poliomyelitis in the developed world.

p0015 Similarly, an outbreak of hepatitis A was observed to be associated with eating raw oysters before the agent had been isolated or vaccines developed. Now, the hepatitis A virus (HAV) is also known to be essentially human-specific and shed in feces, and oysters as well as other foods such as dried tomatoes or frozen berries have been implicated in outbreaks of foodborne viral infections worldwide. A global burden of foodborne disease estimate was published under the auspices of the World Health Organization. Based on systematic literature review combined with modeling and analysis of the proportion of cause-specific disease that could be attributed to contaminated food consumption, the study concluded that viruses are among the top causes of foodborne disease globally (Havelaar et al., 2015). By far the most important disease burden is caused by noroviruses (NoV), but HAV infections are considered important due to the potential severity of disease. Combined, these viruses caused an estimated 138 million cases of foodborne illness in 2010, some 22% of the total global number of foodborne illness episodes. Zoonotic hepatitis E is increasingly recognized as a potential foodborne health threat. The importance of the growing list of other viruses recognized as diarrheal disease agent remains to be evaluated.

* Chapter originally produced by D.O. Cliver (deceased) and S.M. Matsui. This version has been revised for the third edition by the listed authors above.

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s0020

14.1.2 SPECIAL COMMON PROPERTIES OF FOODBORNE VIRUSES

p0020 Viruses are highly evolved microorganisms that multiply (replicate) only within suitable living host cells. This means that viruses cannot replicate in food or in the environment. The particulate forms in which the viruses are transmitted are too small to be visible even with microscopes that can view bacteria, so their appearance remained a mystery until the electron microscope was invented. Many important features of virus structure are still under investigation. A virus particle may consist of either RNA or DNA that is single or double stranded, monopartite or segmented, surrounded and protected by a protein coat, and sometimes an outer envelope of lipid-containing material. Most of the foodborne viruses contain RNA (usually single stranded) and lack an envelope outside their protein coats (Table 14.1). In addition to the classification criteria shown in the table, each virus group (genus) has its own characteristic genetic organization.

p0025 To infect susceptible cells, the viral particle must encounter an appropriate receptor on the plasma membrane of a potential host cell to begin the infectious cycle. Most viruses transmitted via foods replicate in the human body and are shed in feces or, more rarely, in vomitus. Viruses are inert particles when outside their hosts, and the risk posed by their presence in food greatly depends on their environmental stability. Human enteric viruses enter the environment through contamination of surfaces or the discharge of sewage-contaminated water. NoVs and HAV can remain infectious for long periods of time on soiled surfaces. For wastewater, the type of treatment before environmental release, if any, will determine the concentration of pathogens and the relative risk of disposal. Viruses are present in high numbers in raw wastewater, and current water treatment practices do not ensure the complete removal of viral pathogens; hence, viruses become environmental pollutants. Consequently, all food that can be in contact with incorrectly treated sewage and then consumed raw may be the source of foodborne outbreaks. Molluscan shellfish, grown in coastal waters potentially exposed to human fecal pollution, are frequent vehicles of viruses. Shellfish may concentrate the virus during their filter-feeding activities

t0010 **Table 14.1** Most Relevant Foodborne Viruses

Common Name	Particle/Genome	Genus	Family
Human norovirus	Nonenveloped/ssRNA	*Norovirus*	Caliciviridae
Human sapovirus	Nonenveloped/ssRNA	*Sapovirus*	Caliciviridae
Human astrovirus	Nonenveloped/ssRNA	*Mamastrovirus*	Astroviridae
Human rotavirus	Nonenveloped/segmented dsRNA	*Rotavirus*	Reoviridae
Human enteric adenovirus	Nonenveloped/dsDNA	*Mastadenovirus*	Adenoviridae
Human parvovirus[a]	Nonenveloped/ssDNA	*Parvovirus*	Parvoviridae
Hepatitis A virus	Nonenveloped/ssRNA	*Hepatovirus*	Picornaviridae
Hepatitis E virus	Nonenveloped/ssRNA	*Orthohepevirus*	Hepeviridae

ds, *double-stranded;* ss, *single-stranded.*
[a]*Rarely or never foodborne.*

and yield it only very slowly under depuration conditions. Selective transmission of some NoV strains to humans via oysters through specific binding to carbohydrate ligands was demonstrated. These ligands that influence both bioaccumulation and persistence of some viral particles in oysters show that oysters are not just filters or ionic traps but that they can specifically concentrate some pathogens.

s0025 14.2 HEPATITIS A VIRUS AND HEPATITIS E VIRUS

s0030 14.2.1 AGENT

p0030 The HAV is the prototype of the genus *Hepatovirus*, within the Picornaviridae family. The particle has a coat of protein around a single strand of plus-sense RNA and has a diameter of about 28–30 nm, with no distinctive surface features. HAV is a highly stable virus even under extreme conditions such as high temperatures, very low pH, or desiccation. However, its sensitivity to genome-target affecting treatments such as UVC light is much higher than that to high temperatures and low pH, which suggests that the highly resistant phenotype of HAV is mainly due to an extremely stable capsid. As a result, HAV in food can withstand processes that would inactivate other viruses and, indeed, most vegetative cells of bacterial pathogens. Despite a nucleotide diversity similar to that of other picornaviruses, capsid structural constraints limit its amino acid variability, and thus HAV exists as a single serotype, with human strains distributed into three genotypes (I, II, and III) and seven subgenotypes (IA, IB, IC, IIA, IIB, IIIA, and IIIB). Available inactivated HAV vaccines, highly effective against all genotypes, provide long-lasting immunity that persists for at least 15 years, mainly based on the induction of high titers of specific and neutralizing antibodies.

s0035 14.2.2 DISEASE

p0035 Hepatitis A is a common form of acute hepatitis and occurs worldwide. Although chronic cases have never been described and its severity is low, compared with hepatitis B and C, clinical impact increases with age of first exposure, with mortality rates of up to 1 per 100 patients in older adults. Hepatitis A is an endemic infection in developing countries, while its incidence is much less frequent in developed countries. The endemicity pattern has important implications for the average age of exposure and, hence, on the severity of the clinical disease. Since hepatitis A infection induces life-long immunity, severe infections among adults are rare in highly endemic regions where most children are infected early in life, usually without clinical symptoms. In contrast, in low endemic areas, the disease occurs mostly in adulthood, developing as a severe acute symptomatic illness.

p0040 The incubation period of hepatitis A ranges from 15 to 50 days, and clinical illness usually does not last longer than 2 months, although 1.5–15% of patients have prolonged or relapsing signs and symptoms for up to 6 months. A high and long-lasting viremia has been reported, with the peak (up to 10^7 genome copies/mL of

sera) occurring from just before the beginning to 2 weeks after the onset of symptoms and the viremia lasting up to an average of 6 weeks after the start of symptoms. Fecal shedding of the virus reaches its maximum just before the onset of symptoms, at which point the individual is most infectious. Occasionally, the infection may proceed to fulminant hepatitis, mainly among patients with underlying chronic liver diseases. Symptoms include fever, loss of appetite, nausea, and abdominal discomfort, often followed after a few days by jaundice. The immune response normally lasts for life. Immunity begins with production of IgM-class antibody, which declines after a few months. This anti-HAV IgM response is usually limited to the initial infection and is used as a marker of acute disease. IgA is also induced for a limited period of time and is present in serum and feces, but the role of the secretory immunity in protection against HAV infection appears to be very limited. In contrast, the IgG response is delayed compared with IgM and IgA responses but is long-lasting and confers resistance to reinfection. Infected cells in the liver are destroyed by the action of cytotoxic T cells.

s0040 14.2.3 TRANSMISSION

p0045 As has been stated, HAV is shed exclusively in feces, so the source is human feces shed during illness or, more likely, during the incubation period or an unapparent infection. However, outbreaks have certainly occurred from food handled by persons who were visibly jaundiced. Direct person-to-person fecal-oral transmission of HAV is perhaps most common and occurs when food is handled by an infected person without proper hygiene practices. Vehicles in outbreaks from this source are most often foods that are not cooked after initial preparation, such as salads and sandwiches.

s0045 14.2.4 NOTEWORTHY OUTBREAKS

u0010 • People who ate raw clams in Shanghai, China, during 1988 year-end festivities experienced an outbreak of hepatitis A, apparently due to sewage contamination of the shellfish beds. Nearly 300,000 people were affected. An outbreak of gastroenteritis, which may have been viral, preceded the onsets of hepatitis.

u0015 • A hepatitis A outbreak occurred in 2013 across nine U.S. states (Arizona, California, Colorado, Hawaii, New Hampshire, New Mexico, Nevada, Utah, and Wisconsin), affecting 154 patients; it was caused by the consumption of contaminated pomegranate seeds from Turkey (http://www.cdc.gov/hepatitis/Outbreaks/2013/A1b-03-31/).

u0020 • A protracted outbreak of hepatitis A across Europe was caused by consumption of berries, resulting in more than 1500 cases linked to this source of infection. As the berries had been frozen, the outbreak could continue over a long period of time (Severi et al., 2015).

s0050 ### 14.2.5 HEPATITIS E VIRUS

p0070 HEV is a nonenveloped, spherical virus of approximately 30–34 nm in diameter with an indefinite surface substructure slightly less pronounced than that of the caliciviruses. The genome is a single-stranded, positive-sense, polyadenylated RNA molecule of approximately 7.5 kb organized in three open reading frames (ORFs). Hepatitis E generally resembles hepatitis A, with three significant exceptions: the incubation period is slightly longer, with a range of 15–62 days and a mean that varies from 26 to 42 days on different occasions; the target age group is generally young adults, rather than children; and infections can be lethal, especially in pregnant women. Peak shedding of HEV occurs during the incubation period and early acute phase of disease. There are four genotypes of HEV infecting humans, two of which (genotype III and IV) are also present in pigs. The HEV genotypes I and II are transmitted from person to person only and have been associated with large outbreaks of predominantly waterborne disease, particularly in regions and conditions of low hygiene, such as refugee camps. They have been linked to unusually severe illness in pregnant women, with a high probability of fulminant hepatitis, which is associated with high mortality (Kmush et al., 2015). Instead, genotype III and IV infections are thought to be zoonotic infections and often are mild or asymptomatic, although severe and occasionally fatal disease is found in persons with underlying health problems. Recent studies have shown a high prevalence of genotype III HEV in commercial pig herds across industrialized countries, and environmental presence of HEV. The exact patterns of transmission of zoonotic HEV remains to be established, but the high rate of asymptomatic infections has triggered debate on the relative contribution of food consumption to the burden of disease associated with the genotype III HEV.

s0055 ## 14.3 NOROVIRUS

s0060 ### 14.3.1 AGENTS

p0075 The original NoV was found in stool samples from a patient in a gastroenteritis outbreak at a school in Norwalk, Ohio, in 1968. In 1990, the genetic make-up and sequence of the Norwalk agent were identified, marking the start of the molecular era for NoV. Since then, NoVs have been identified as the most common viral cause of gastroenteritis in people of all age groups. The viruses have been assigned to the calicivirus family, which also includes another genus of diarrheal agents, *Sapovirus*. Caliciviral particles have a single molecule of positive-strand RNA coated with many copies of a single capsid protein. The diameter is about 27–28 nm, and the surface has a defined structure on electron microscopy. Many strains of NoV, constituting at least six genogroups, are known in humans and a range of animals, including cattle, pigs, mice, dogs, cats, porpoise, and bats, but humans are mostly infected by viruses belonging to genogroups (GGs) I and II, and particularly GGII.3 and GGII.4. Since the 1990s, GGII.4 viruses have been dominant, and these viruses evolve rapidly

through mutation. As a consequence, every 2 to 3 years, a new (drift) variant emerges, which escapes the antibodies blocking infection that arose from prior infections. In outbreaks, GGII.4 is most often seen as causes of outbreaks in healthcare settings, whereas a broader diversity of viruses is found in foodborne outbreaks. The human caliciviruses are essentially human specific; no easy-to-use in vitro cell culture host or animal model has yet been identified, but recent developments suggest that cell culture models will become available in the near future. It is important to answer outstanding questions about the stability of NoV in food under different processing conditions but also to understand essential questions about population level immunity, whether infection with one NoV may protect from subsequent infections among others (De Graaf et al., 2016).

s0065 14.3.2 DISEASE

p0080 The disease is marked by a 12- to 48-h incubation period (mean 36 h), followed by nausea, vomiting, and diarrhea, although various aches and low-grade fever may also occur. The illness is usually over in 12–60 h in persons with normal health, but fecal shedding of the virus sometimes continues for more than 1 week. Recent studies have shown that more severe illness occurs in persons with underlying health problems, leading to chronic diarrhea and prolonged virus shedding. In addition, mortality peaks have been linked with seasonal peaks of NoV outbreaks, mostly due to excess mortality in the elderly, similar to what has been observed for influenza viruses.

p0085 During illness, intact viral particles may be found both in the diarrheal stool and in the vomitus. The current state-of-the-art diagnostic approach is based on detection of the viral genome, and the amount of virus shed during the acute stage of illness has been estimated to be up to 10^{10} particles per milliliter or gram of stool. Infection leads to production of antibody against the virus, but immunity apparently does not last much more than 1 year. In addition, NoVs evolve rapidly through mutation and recombination and can escape the antiviral activities of antibodies triggered by prior exposures to viruses belonging to the same genotype.

s0070 14.3.3 TRANSMISSION

p0090 Only small numbers of NoV particles are required to cause infection (less than 100 particles). They can pass through groups (e.g., children in daycare centers) very rapidly. Fecal shedding, again, occurs during illness and sometimes continues for 1 week or longer. In chronic infections, this can be years, although there currently is very little evidence that chronic shedders contribute to spread of infections. Predominant vehicles for virus transmission are bivalve filter feeding mollusks and uncooked, ready-to-eat foods. Because these viruses are very contagious and immunity is not durable, attack rates in common-source outbreaks tend to be high. The NoVs often produce secondary cases—those who have been infected by eating contaminated food pass their infections to other people who have not eaten the food. Due to this high secondary attack rate, finding the source of an outbreak may be difficult.

s0075 ### 14.3.4 NOTEWORTHY OUTBREAKS

u0025 • In 1982, a baker's assistant who worked at a large bakery in the vicinity of Minneapolis and St. Paul, Minnesota, prepared a large batch of butter-cream frosting while experiencing diarrhea. The frosting was served on various pastries and transmitted Norwalk gastroenteritis to at least 3000 patrons. Although the individual illnesses were brief, large numbers of teachers and hospital staff were affected, which significantly disrupted activities at local schools and hospitals.

u0030 • A North Yorkshire, UK, cook vomited into a sink and cleaned it out with a sanitizing agent that was used in the restaurant where he worked. The sink was used to prepare potato salad the next day, and at least 47 people who ate the salad at a wedding reception contracted viral gastroenteritis.

u0035 • The Duke University (North Carolina) football team ate a box luncheon and then flew to Florida State University (Tallahassee, Florida) for a game the next day. Of those who ate the box lunch, at least 43 (62%) were stricken with NoV gastroenteritis—many during the game. Another 11 people among the Duke party and 11 members of the Florida State team became ill the following day as secondary cases. The two teams had no contact off the playing field and had shared no food or beverages.

u0040 • In 1993, a multistate outbreak of NoV gastroenteritis was traced to oysters harvested from a bed near where an ill oyster harvester had disposed of contaminated raw sewage. Despite a large dilution factor, the harvested oysters contained enough NoV to transmit disease. Sequence analysis of the virus found in infected individuals linked the outbreaks with the contaminated oysters.

u0045 • A huge outbreak in 2012 involving more than 11,000 cases occurred in Germany, through consumption of frozen strawberries.

s0080 ## 14.4 OTHER GASTROENTERITIS VIRUSES

s0085 ### 14.4.1 AGENTS

p0125 Two additional groups of viruses that cause food-associated gastroenteritis are the astroviruses (AstVs) and the rotaviruses (Table 14.1). The AstVs are about the size of the NoV and picornaviruses but are distinguished by a characteristic star-like surface appearance (*astron* means "star" in Greek). The AstV genome is a positive-sense single-stranded RNA molecule of around 6.8 kb excluding the polyadenylated tail at the 3' end. A VPg protein is covalently linked to the 5' end of the genome. The genome contains three open reading frames. Although initially detected in children's stools, AstVs have been found in the feces of a wide variety of mammalian species: cats, cattle, deer, dogs, mice, rats, pigs, sheep, mink, bats, cheetahs, rabbits, and even sea lions and dolphins, as well as in avian species

such as turkeys, chickens, ducks, pigeons, guinea fowl and other wild aquatic birds. Human AstVs were initially distributed in eight serotypes and they have been adapted to growth in cell culture. However, the complete picture of the AstV field has dramatically changed with the discovery after metagenomic surveillance studies of a variety of highly divergent novel AstVs able to infect different animal species, including humans, which are unrelated to the previously described eight serotypes of HAstVs, now termed classic HAstVs. The first novel HAstVs were identified in 2008 in pediatric stool specimens in Melbourne, Australia. They were termed HAstV-MLB, and so far several MLB-related strains (named MLB1, MLB2, and MLB3) have been detected in different parts of the world. In 2009, a second group of novel HAstVs was described in samples from children with diarrhea in Virginia, USA, and in Nigeria, Pakistan, and Nepal (HMO, referring to human, mink and ovine-like Asts).

p0130 The rotaviruses contain double-stranded RNA that is divided into 11 segments. The coat protein comprises a double layer of 70–75 nm in diameter, the outermost of which must be removed by proteolysis before the viral particle can enter a host cell. The particle is perceived as looking like a wheel ("rota" in Greek) in electron micrographs. Many serotypes, assigned to eight groups are known. The majority of human illnesses appear to be caused by members of group A, but common-source outbreaks may more often involve members of other serogroups. There are human and animal strains of *Rotavirus*; human and animal reassortant viruses (containing certain RNA segments from one parent virus and other RNA segments from the other parent virus) may cause human infection and contribute to the diversity of *Rotavirus* infection. Human rotaviruses found in clinical specimens may be propagated in cell culture although with difficulties.

s0090 14.4.2 DISEASES

p0135 AstVs, especially classic AstVs, are considered gastrointestinal pathogens affecting children worldwide, with very few reports of HAstV-mediated disease in normal healthy adults. Symptoms are similar to those of NoV infection, although vomiting is less common. AstVs also appear to cause a milder illness with less dehydration than seen with *Rotavirus*. Immunity is probably relatively durable, although outbreaks have been reported in populations expected to be immune (see Section 14.4.4). Infections caused by the other novel types of nonclassic HAstVs (HAstV-MLB and HAstV-VA/HMO) are generally associated with gastroenteritis as well, but their pathogenic role in human health has not been clearly elucidated. Additionally, extraintestinal dissemination in immunodeficient individuals has also been observed in HAstV-HMO C (VA1) virus infections, when the agent was detected in neural tissue from an immunocompromised child with encephalitis.

p0140 Rotaviruses frequently affect young children throughout the world. In developing countries, they are a significant cause of child mortality. The illness involves vomiting, fever, and watery diarrhea beginning after 24–72 h of incubation and lasting 4–6 days, on average. Shedding is said seldom to continue beyond the eighth day

of infection, except in the immune impaired. A recent study from Japan indicates that symptomatic *Rotavirus* infection occurs commonly among healthy adults, challenging the presumption of durable immunity. Since the introduction of *Rotavirus* vaccines, the number of *Rotavirus* hospitalizations has decreased greatly in countries with high vaccine coverage.

s0095 ### 14.4.3 TRANSMISSION

p0145 Both the AstVs and the rotaviruses are mostly transmitted by a fecal-oral route with vehicles such as food and water occasionally involved. Although person-to-person transmission may predominate, vehicular outbreaks have been recorded on various occasions.

s0100 ### 14.4.4 NOTEWORTHY OUTBREAKS

u0050 • Contaminated food from a common supplier apparently transmitted AstV to more than 4700 students and teachers at 10 primary and four junior high schools in Katano City, Osaka, Japan, in June 1991.

u0055 • Eating delicatessen sandwiches in a university cafeteria in the District of Columbia evidently led to *Rotavirus* gastroenteritis in at least 108 students in March and April 2000. Two cooks at the dining hall were ill, but their illnesses occurred during the outbreak, rather than before it.

s0105 ## 14.5 OTHER VIRUSES AND FOOD

s0110 ### 14.5.1 TICK-BORNE ENCEPHALITIS VIRUS

p0165 The tick-borne encephalitis (TBE) virus shows that foodborne transmission is not limited to viruses without envelopes. The virus is enveloped with a lipid-containing layer and contains RNA. A tick biological vector transmits the virus to dairy animals—principally goats, given the terrain, but also sheep and cows, in regions with enzootic presence of TBE. Virus shed in the milk of these animals may infect humans, if they drink the milk unpasteurized, and cause a serious form of encephalitis. Outbreaks are rare since TBE vaccination has been introduced in regions where the virus is present.

s0115 ### 14.5.2 OTHER ENTERIC VIRUSES THAT MAY BE FOODBORNE

p0170 Several other kinds of human viruses are transmitted by the fecal-oral route (though they are not primarily associated with gastroenteric syndromes) and might thus be transmitted via food or water. The three types of viruses that caused poliomyelitis (polioviruses), which has now been eradicated in much of the world by vaccination, are members of a larger group of human enteroviruses. The enteroviruses are members of the picornavirus family and include, in addition to the polioviruses, other

groups called coxsackieviruses A and B and echoviruses, as well as enterovirus types with numbers after their names. These cause a variety of illnesses, including meningitis and encephalitis; members of the various groups have been implicated in rare outbreaks of foodborne illness. The adenoviruses contain double-stranded DNA and show a classic icosahedral shape. Most adenoviruses are associated with respiratory infections, but serotypes 40 and 41 cause gastroenteritis in humans and are apparently transmitted by the fecal-oral route. Food-associated adenovirus outbreaks are apparently unknown.

s0120 14.6 DETECTION AND MONITORING

p0175 Given that viruses are significant causes of foodborne disease, there is a need for virus detection methods in food. This presents special problems because viruses are likely to be present at low levels (yet still a threat to consumer health) and heterogeneously distributed in food, and cannot be enriched from a sample, as bacterial pathogens often are. Furthermore, the most important of the foodborne viruses, HAV and NoVs, cannot easily be grown in cell culture to demonstrate infectivity. Progress has been made in detection methods for food, although most outbreaks of foodborne virus disease are recognized by diagnosing the infection among humans.

s0125 14.6.1 SAMPLING AND SAMPLE PROCESSING

p0180 Samples are selected for testing, either because of a general perception of hazard or because a food history has implicated these particular foods in an outbreak that is under investigation. Because of the long incubation period of hepatitis A (average 4 weeks), foods representative of what was eaten that led to a hepatitis outbreak may well be unavailable for testing by the time the etiology is established. Otherwise, the most common problem with samples from outbreak investigations is that they have been exhausted in bacteriological testing or allowed to deteriorate badly before virological testing was considered. The size of the sample may be governed by what is available but also by the test method that is to be used. The choices, all largely adaptations of methods that have been used to diagnose viral diseases, include transmission electron microscopy, cell culture infectivity, serological reactions, and reactions with the viral nucleic acid (so-called molecular methods) or combinations of these.

p0185 Since no enrichment of virus can be done, it is important that samples be extracted carefully (to separate virus as completely from food components as possible) and then concentrated with minimum loss of virus. Samples in various studies have usually ranged from 10 to 100 g, largely on the basis of expediency.

s0130 ## 14.6.2 AVAILABLE DETECTION METHODS

p0190 Electron microscopy was in the past an important tool in diagnosis and characterization of viral diseases. In addition to lack of sensitivity for testing food and water samples, it also suffers from the limitation that a great variety of viruses have similar morphology. This can sometimes be remedied by performing immunoelectron microscopy using a known antiserum to collect or agglutinate the viruses on the electron microscope grid.

s0135 ### *14.6.2.1 ISO/CEN * Method, Qualitative, and Quantitative Detection of NoV and HAV*

p0195 A standardized reference method for molecular detection of NoV and HAV in different food matrices such as bivalve mollusks, leafy green vegetables, berries, food surfaces, and bottled water was published in 2016 (ISO/TS15216). The standard describes matrix-specific virus extractions and a common RNA capture method based on magnetic silica capture followed by detection using real-time one-step reverse transcription–polymerase chain reaction (RT-PCR) with a fluorescence probe under a qualitative or a quantitative assay (parts 1 and 2). For bivalve mollusks after dissection of 2 g digestive tissues and digestion using a proteinase K protease, nucleic acids are extracted from a part of the supernatant for further purification and detection. For other foods such as leafy green vegetables or berries, viruses are eluted from 25 g of the food surface and then concentrated by polyethylene glycol concentration. For bottled water, viruses are recovered by filtration and for food surfaces by swabbing. As virus detection in food matrices is challenging due to physical and chemical properties of the food, this method includes a number of controls to prevent false-negative results or underestimation of virus quantity. A virus (that cannot be present in any food matrix) has to be added as an external process control in order to measure the virus extraction efficiency. The inhibition of target amplification is evaluated by adding an RNA control to the real-time RT-PCR. This method, targeting the two major viruses and food matrices implicated in outbreaks worldwide, is the first international recognition of an ISO method for virus in food. The various controls and detailed protocols, selected from peer review publications, optimized, and validated, increase confidence in the results and their interpretation. However, using a standardized method also has some disadvantages as it lacks flexibility befitting such genetically versatile pathogens. Also the high number of controls increases costs and the lack of reference materials such as reference plasmids for standard curves has to be solved.

p0200 Quantification is important to better understand clinical disease in food consumers and for routine monitoring as it can provide data to develop acceptance levels in food commodities and quantitative risk assessments. Quantification by real-time RT-PCR can be done by using a standard curve generated from known amounts of the target sequence represented by synthetic or in vitro transcribed RNA or DNA, although this process needs to be carefully validated, as it is error prone. Moreover, the viruses are

* ISO: International Standardization Organization; CEN: Commission for European Normalization

often unevenly distributed, which makes it necessary to test replicates or a pool of samples (bivalve mollusks) to obtain the most reliable qualitative or quantitative results.

p0205 To confirm detection of a positive signal and to assist epidemiological studies, strain typing is recommended. For this purpose, sequencing a small genome fragment has been recommended, although this practice is likely to change in the near future with the rapid development of next-generation sequencing technology. However, sequences are difficult to obtain from food samples and less than 35% of positive samples can be confirmed by sequencing, showing there is room for improvement.

s0140 ### 14.6.2.2 Infectivity

p0210 The original detection methods for foodborne viruses were generally based on the infectivity of the viruses for cell cultures. Eventually, it became clear that the viruses most often transmitted were not detectable by available cell culture types, but the quest for susceptible cell lines continues. Cell culture can be used to detect enteric viruses that are cultivable, but this requires virus concentration methods that maintain virus viability. Such methods were used for enterovirus and HAV, as some strains can be multiplied in vitro. However, despite numerous attempts on a large number of cell lines, as well as three-dimensional tissue structure using a variety of cell lines, no in vitro replication could be obtained for NoV. Most recently, however, the replication of a GII.4 Sydney NoV strain was demonstrated in B-cells in the presence of histoblood group antigen-expressing enteric bacteria. Human intestinal enteroids were recently demonstrated as a good model for the replication of some *Rotavirus* strains and, thus, could be useful for studying other human enteric viruses. Some methods, such an integrated cell culture RT-PCR, propose a combination of initial amplification by cell culture and then detection by PCR. This has been proposed for the detection of adenovirus, AstV, enterovirus, or HAV.

p0215 The method allows for the detection of infectious HAV in mussels, but other applications are still rare. Indeed, if this technique is more sensitive than cell culture alone, the toxicity of the food matrix for cells and the level of sensitivity may be problematic for routine applications.

s0145 ### 14.6.2.3 Detection of Intact Virus Capsids

p0220 Other approaches can provide information on the integrity of the capsid such as RNase or propidium monoazide treatments, as successfully demonstrated on HAV submitted to thermal inactivation. However, such approaches have to be adapted depending on the virus and treatment applied. Nucleic acid aptamers for the capture of some NoV strains have been proposed, and single-stranded DNA aptamers may be a good surrogate to antibodies. Other techniques, such as the use of phage nanoparticle reporters in a lateral-flow assay, seem to be promising.

p0225 Based on the capacity of NoVs to recognize histoblood group antigen glycans, these glycans have been proposed as tools for the evaluation of capsid integrity. After treatment by chlorine, heat or UV radiation, selective binding to glycans showed a 3-log reduction in genomic titers, demonstrating the capacity to specifically target undamaged capsid. This technique was also efficient for evaluating high hydrostatic

pressure on two animal calicivirus strains (the murine NoV and the Tulane virus). The combination of pig mucin binding and RNase treatment reduced detection of damaged particles after different inactivation treatments.

s0150 #### *14.6.2.4 New Developments: Technology*

p0230 Recent technical developments open opportunities for rethinking analyses of food matrices. Beside some technical improvements of quantification as provided by digital PCR (dPCR), accuracy will be enhanced by improvement of enzymes, probe labeling, but also viral genome knowledge. The dPCR is based on the partitioning of the sample into individual standard PCRs carried out on microfluidic chips or microdroplets. The signal of each amplification reaction, containing "in theory" one or no copies of the nucleic acid target, is measured and the absolute number of target molecules are directly calculated, with no need for a standard curve or any reference material. The performance of this technique was found more tolerant to inhibitory substances for the quantification of NoVs and HAV on seeded lettuce and bottled water. This technique was also found interesting for water samples analysis, showing a promising range of applications. On a more general approach, the application of next-generation sequencing to viral genome identification will provide new data that will help to improve primer and probe selection. Some technical developments on identification of the microbiome and virome in clinical and environmental samples will be helpful in future analysis of food or environmental samples.

s0155 ### 14.6.3 PROSPECTS FOR MONITORING VIA INDICATORS

p0235 Since viruses transmissible via foods are typically shed with feces, it is reasonable to try to circumvent the problems of detecting foodborne viruses by instead seeking indicators of fecal contamination. Indicators explored for this purpose have included bacteria, human viruses, and bacteriophages. Since foodborne viruses are human specific, the indicator should show that fecal contamination of human origin is present, but this is seldom possible. Bacterial indicators range from coliforms, fecal (thermotolerant) coliforms, and enterococci to *Escherichia coli* or *Bacteroides fragilis*. The greatest problem with bacteria as indicators is that they do not die at the rates of virus inactivation, and they may even multiply in the environment (including food), so their correlation with the presence of virus has been found to be very poor.

p0240 The bacteriophages considered as indicators include those that infect *E. coli*–related bacteria (coliphages), either via receptors on the cell wall (somatic coliphages) or on the F-pilus (and happen incidentally to contain single-stranded RNA–FRNA (F denotes Flagella) coliphages, as do most foodborne viruses), as well as phages that infect *B. fragilis*.

p0245 Finally, human enteroviruses, such as the vaccine polioviruses, are often present in community sewage and are specific indicators of human fecal contamination, but they present their own detection problems, are unlikely to be present in feces from a single individual who contaminated food during handling, and, in the case of polioviruses, are disappearing with the switch to inactivated vaccine in the end stage of the poliovirus eradication program.

s0160 ## 14.7 PREVENTION

s0165 ### 14.7.1 PREVENTING CONTAMINATION OF FOODS

p0250 Since viruses transmitted via foods are shed almost exclusively in human feces (with the minor exception of the NoVs shed in human vomitus), preventing contamination is simply a matter of keeping human feces out of food. Where contamination via water (e.g., of shellfish) is concerned, the challenge begins with the fact that our basic standard of community hygiene calls for water-carriage toilets. Every time a toilet is flushed to dispose of feces, a considerable volume of water becomes contaminated; when this sewage reaches the treatment plant, it is a challenge to remove or inactivate any virus that may be present before the wastewater is discharged to be used for another purpose or to enter an estuary in which mollusks are growing. Food contamination via feces-soiled hands is preventable by hand-washing, but the necessary cleaning does not always take place, owing to carelessness, cultural blocks, or unavailability of water for this purpose. The most extreme case is outbreaks of mollusk-associated viral disease where the waters were contaminated by direct fecal discharge from the harvesters. The mode of fecal contamination in the recent outbreak involving frozen strawberries has never been demonstrated. For HAV, vaccination can be considered to prevent foodborne disease.

s0170 ### 14.7.2 INACTIVATION OF FOODBORNE VIRUSES

p0255 Viruses cannot multiply in food, like some other pathogens. Therefore, the viruses can only persist or be inactivated before the food is eaten. Virus on food surfaces or in water is accessible to chemical inactivation by strong oxidizing agents (e.g., chlorine) or by UV light. HAV withstands drying on surfaces reasonably well, but many other viruses are inactivated by drying. Virus within a food ordinarily can only be killed by heat: although ionizing radiation penetrates food well, the target size of viruses is so small that relatively large and costly irradiation doses are required. Viruses are relatively stable under frozen and chilled storage conditions and will gradually lose infectivity at any temperature above freezing, but inactivation of viruses within the shelf-life of most foods requires elevated temperatures. The heat resistance of most viruses is little greater than those of many nonsporing bacterial pathogens transmissible via foods.

s0175 ## 14.8 SUMMARY

p0260 Viruses are frequent causes of foodborne disease globally. Most of the foodborne viruses are shed in human feces and infect perorally. The great majority of these are RNA viruses, often small and containing single-stranded RNA. They infect via the intestinal lining, but some are transported to the liver (and occasionally other organs) before causing disease. Virus particles are submicroscopic in size and cannot multiply in foods. They have been known to contaminate foods at levels that

caused thousands of cases, but most foodborne virus transmission probably results from contamination of small quantities of food, eaten by small numbers of people, by feces on unwashed human hands. It is often possible to detect viruses in food samples, but the difficulty is such that one must have a compelling reason for conducting the test. Washing hands and cooking foods are probably the two measures that contribute the most to preventing foodborne viral disease.

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Non-Print Items

Abstract
Some diseases now known to be viral were recorded as foodborne before the nature of viruses was understood. In particular, outbreaks of poliomyelitis associated with drinking raw milk were recorded before the poliomyelitis viruses (polioviruses) had been isolated. Polioviruses only infect humans and other primates and are shed in feces. The problem of milk transmission of polioviruses, therefore, was a clear example of food handler–associated transmission, which was solved by improved sanitation and almost universal pasteurization of milk before vaccines became available and eradicated poliomyelitis in the developed world.

Keywords: Gastroenteritis viruses; Hepatitis A virus; Hepatitis E virus; Noroviruses; Viruses.