CHAPTER 13

Survival of Enteric Viruses in the Environment and Food

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1. INTRODUCTION

Enteric viruses are those human viruses that are primarily transmitted by the fecal-oral route, either by person-to-person contact or by ingestion of contaminated food or water. The importance of viral foodborne diseases is increasingly being recognized, and several international organizations have found that there is an upward trend in their incidence. Consequently, there is a growing concern over human exposure to enteric viruses through contaminated food products. Data on viral foodborne diseases are still fragmented, and epidemiological studies have focused either on particular countries or on particular pathogens. In the last decade, epidemiological reports indicate that enteric viruses, in particular human noroviruses (NoV), which cause acute gastroenteritis, and hepatitis A virus (HAV), are the leading cause of foodborne illness in developed countries (Koopmans and Duizer 2004; EFSA 2015). Other enteric viruses, including rotaviruses, sapoviruses, astroviruses and hepatitis E virus (HEV), are not frequent causes of foodborne disease but can occasionally be transmitted by contaminated foods.

In numerous NoV or HAV cases, the vehicle(s) of virus spread and food contamination remains unidentified. Much epidemiological evidence suggests that infected food handlers and contaminated food-contact surfaces may play an important role in food contamination. Food may become contaminated with enteric viruses either by fecal contamination, cross contamination from another food product, or by an infected food handler. While consumption of ready-to-eat foods contaminated by infected food handlers remains the major risk factor for viral foodborne outbreaks, many types of food products are being recognized as vehicles of viruses in causing gastroenteritis or hepatitis A outbreaks (Table 13.1).

In the EU, foodborne viruses (mainly human NoVs) were identified to be the most frequently detected causative agents of foodborne outbreaks in 2014, accounting for 20.14 % of the reported outbreaks (EFSA 2015). In 2011, the Centers for Disease Control and Prevention (CDC) issued new figures for the incidence of foodborne illness, estimating that about 5.5 million people in the USA suffer from viral foodborne illnesses each year, resulting in 15,300 hospi-

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talizations and 156 deaths. Moreover, the cost of foodborne illness in the U.S.A. is now estimated to be around $3000 million a year according to a study conducted by the Ohio State University (Scharff 2012).

As a reflection of the seriousness of viral foodborne outbreaks, extensive attention has been given to them by national and international organizations over the last 10 years. Examples of which include: the report of the Advisory Committee on the Microbiological Safety of Food, the recently proposed guidelines for the application of food hygiene to the control of viruses for Codex Alimentarius (CX/FH/10/42/5), the scientific opinion of the European Food Safety Authority (EFSA) (http://www.efsa.europa.eu/fr/efsajournal/pub/2190), and the expert advice on foodborne viruses for Codex Alimentarius (www.who.int/foodsafety/publications/micro/mra13/en/index.html). This latter document concluded, among other considerations, that prevention and control measures should be considered for enteric viruses in bivalve molluscan shellfish, fresh produce, or prepared foods.

Virus persistence Because viruses outside their hosts are inert particles, their chances of transmission from host to host are greatly dependent on the degree of their robustness, which allows them to remain infectious during the various conditions they encounter in the environment and foods. Numerous physical, chemical, and biological factors influence virus persistence in the environment (Table 13.2). Some of the primary factors affecting the survival of viruses in liquid environmental matrices are temperature, ionic strength, chemical constituents, microbial antagonism, the sorption status of the virus, and the type of virus. Considerable differences have been observed in the survival of enteric viruses in different types of environmental and food samples. Different behaviors and inactivation rates have been observed not only among viruses of different families and genera, but also among viruses of the same family, genus, and even among similar types or strains of virus. Among the chemical constituents of liquid or semisolid (feces, human night soil, biosolids, and animal manures, etc.) environmental matrices, the amount and type of organic matter and specific antiviral chemicals (such as ammonia at elevated pH levels or natural antimicrobial compounds of fruits) play a role in virus stability. Of the physical factors influencing virus persistence in liquid media, temperature, sunlight, and virus association with solids are among the most important. Soil moisture, temperature, sunlight, and other soil characteristics may influence the persistence of viruses in soil. On inanimate surfaces (or fomites), the most important factors that affect virus stability are the type of virus and surface, relative humidity, moisture content, temperature, composition of the suspending medium, light exposure, and presence of antiviral chemical or biological agents. Most of these factors are also relevant for the ability of viruses to persist in aerosolized droplets, together with the moisture content and the size of the aerosol particles, and the air quality.

Understanding food and environmental virus stability, and elucidating the factors that affect it, may shed some light on the potential public health risk associated with these viruses and at the same time provide tools to interrupt the chain of fecal-oral transmission. In this chapter, only studies involving the

Table 13.1 Selected large outbreaks (over 100 cases) of hepatitis A virus and norovirus occurring in the last 10 years

Year	*Location*	*Implicated food*	*Responsible virus*	*Raw material origin*	*Number of cases*	*Reference*
2012–2013	Nordic European countries	Frozen strawberries	HAV		103	Nordic outbreak investigation team 2013
2012	Germany	Frozen strawberries	NoV	China	>11,000	http://www.bfr.bund.de/en/norovirus_outbreak_2012-186684.html
2011	US	Pomegranate seeds	HAV	Turkey	162	http://www.cdc.gov/hepatitis/Outbreaks/2013/A1b-03-31/index.html
2010	Denmark	Lettuce	NoV	France	260	Ethelberg et al. (2010)
2009–2010	Australia, The Netherlands, France	Sun dried tomatoes	HAV	Turkey	308	Gallot et al. (2011), Petrignani et al. (2010)
2008	China	Bottled water[a]	HAV	China	269	http://www.china.org.cn/government/local_governments/2008-04/24/content_15007889.htm
2007	France	Oysters	HAV		111	Guillois-Bécel et al. (2009)
2005	Denmark	Frozen raspberries	NoV	Poland	>1000	Falkenhorst et al. (2005)
2004	Belgium	Raw beef[b]	HAV		269	Robesyn et al. (2009)
2004	Egypt	Orange juice[c]	HAV		351	Frank et al. (2007)
2003–2004	Singapur	Oysters[d]	NoV		305	Ng et al. (2005)
2003	USA	Green onions	HAV	Mexico	601, 3 deaths	Wheeler et al. (2005)

[a]Suspected contamination of the water source with runoff from melting of heavy snowfall
[b]Food handler contamination
[c]Poor hygiene during processing
[d]Frozen and served thawed

Table 13.2 Factors affecting virus persistence in the environment

Factor	*Effect*
Physical	
Heat	Inactivation is directly proportional to temperature
Light	Light, especially its UV component, is germicidal
Desiccation or drying	Usually inactivation increases at lower relative humidity
Aggregation/adsorption	Protection from inactivation
Pressure	High pressure induces inactivation
Chemical	
pH	Worst stability at extreme pH values
Salinity	Increased salt concentrations are virucidal
Ammonia	Virucidal
Inorganic ions	Some (e.g. Ag, Pt, Pd, Rh) are virucidal
Organic matter	Dissolved, colloidal, and solid organic matter protect from inactivation
Enzymes	Proteases and nucleases contribute to inactivation
Biological	
Microbial activity	Contributes to inactivation
Protozoal predation	Contributes to removal/death
Biofilms	Adsorption to biofilms protects from inactivation, while microbial activity in biofilms may be virucidal
Type of virus	Stability varies according to the strain and type of virus

persistence of enteric viruses in the absence of any deliberately applied inactivation process are reviewed. Neither work on virus disinfection nor studies conducted with potential indicators, such as bacteriophages, are considered.

2. METHODS TO STUDY VIRUS PERSISTENCE IN FOOD AND THE ENVIRONMENT

Most studies to determine the potential of enteric viruses to persist in food matrices or in the environment have been performed by artificially adding a known amount of virus to a given sample, determining the reduction in the infectious titer after subjecting the spiked sample to designated conditions, and applying statistical procedures to determine the significance of virus decay. Obviously, this implies the use of virus strains that may be propagated in cell cultures and enumerated through infectivity, thus greatly restricting the range of viruses that are able to be included in these studies.

This is extremely relevant for NoV, since although attempts to culture them have been made (Guix et al. 2007; Straub et al. 2007; Papafragkou et al. 2013; Jones et al. 2014), research with human NoV has been hampered by the lack of suitable laboratory animals and the inability to propagate the virus in vitro. Consequently, the use of surrogates including feline calicivirus (FCV), murine norovirus (MNV), and Tulane virus (TV) is being extensively used to evaluate the NoV persistence in the environment and different food matrices.

Moreover, in the last decade molecular detection approaches such as RT-PCR or real-time quantitative RT-PCR (RT-qPCR) are normally employed for fastidious virus analysis. However, these techniques are unable to differentiate between infectious and non-infectious particles and, therefore, unsuitable for virus persistence studies. A promising approach to avoid this drawback relies on the use of nucleic acid intercalating dyes such as propidium monoazide (PMA) or ethidium monoazide (EMA) as a sample pretreatment previous to the RT-qPCR. So far, PMA combined with RT-qPCR has successfully been applied to discriminate between infectious and heat-treated non-infectious viruses e.g., poliovirus, coxsackievirus, echovirus and HAV (Parshionikar et al. 2010; Sánchez et al. 2012; Coudray-Meunier et al. 2013). Moreover, EMA has also been used to distinguish between thermally inactivated MNV and poliovirus suspensions (Kim and Ko 2012).

Other alternative strategy to increase the likelihood of detecting intact and potentially infectious viruses is to pretreat the virions with nucleases and/or proteolytic enzymes prior to nucleic acid extraction, amplification, and detection, thereby eliminating the detection of free nucleic acids or nucleic acids associated with damaged, inactivated virions (Nuanualsuwan and Cliver 2003).

Some other health significant enteric viruses, such as rotavirus, astrovirus, and enteric adenovirus, replicate poorly in cell cultures; yet their persistence may be evaluated by integrated cell culture RT-PCR assays (Pintó et al. 1995; Reynolds et al. 1996; Abad et al. 1997; Reynolds et al. 2001). For this purpose, cells supporting the propagation of a wide variety of enteric viruses, such as CaCo-2 (colonic carcinoma) or PLC/PRF/5 cells (human liver hepatoma), have been used as an *in vivo* amplification step prior to molecular amplification (Grabow et al. 1993; Pintó et al. 1994).

Another issue to be considered from an experimental point of view is how the survival experiments are designed. Most studies are performed by artificially adding a known amount of virus to a food sample without considering all the mechanisms of attachment involved. For instance, enteric viruses bind to food products by a variety of mechanisms, including ionic and hydrophobic interactions, van der Waals forces, interaction with receptors (e.g. NoV binding to carbohydrates) and uptake into bivalve mollusk and vegetable tissues, which may have an impact on its survival on these food items.

Moreover, most of the methods for virus detection in food include a key elution step to release the viruses from the food surface, because it is assumed that naturally contaminated samples carry virus particles only on the surface. However, enteric viruses can also attach to the leaf surface and internalize

through stoma and cuts on the leaf during direct contact with contaminated water (Wei et al. 2011). Furthermore, a new mechanism of HAV contamination of green onions was proposed by Chancellor et al. 2006. In this study HAV particles were found trapped inside growing green onions taken up intracellularly through the roots, even though survival of the virus was not evaluated. This mechanism has also been described for NoV and NoV surrogates such as MNV, Tulane virus, porcine sapovirus and canine calicivirus on lettuce (Dicaprio et al. 2012; Esseili et al. 2012; Urbanucci et al. 2009; Wei et al. 2011) and green onions and spinach (Hirneisen and Kniel 2013). This mechanism will definitely change future approaches for the detection of viruses in vegetables, as well as the design of survival experiments in vegetables.

3. VIRUS PERSISTENCE IN THE ENVIRONMENT

Persistence or stability are the terms of choice to describe the capacity of a given virus to retain its infectivity in a given scenario. One critical question in environmental virology is whether or not viruses can persist long enough, and in high enough concentrations in the environment, to cause disease in individuals who are in contact with polluted recreational water, soil, fomites, or contaminated hands.

Some enteric virus infections follow a seasonal pattern, whereas others fail to do so. In regions with temperate climates, infections due to enteroviruses generally reach a peak in summer and early fall. On the contrary, rotavirus, NoV, and astrovirus infections occur mainly during the cooler months (McNulty 1978; Mounts et al. 2000; Guix et al. 2002), although seasonal and non-seasonal distributions of rotavirus in sewage have been described (Hejkal et al. 1984; Bosch et al. 1988). On the other hand, cases of hepatitis A do not show a clear seasonal pattern (Lemon 1985), whereas enteric adenovirus infections are reported to peak in midsummer (Wadell et al. 1989). These data suggest that temperature, and probably relative humidity, may be meaningful in the seasonal distribution of outbreaks of certain human enteric viruses (Enright 1954), due to the influence of these factors on virus persistence.

3.1. Virus Persistence in Environmental Waters

The survival of viruses in environmental waters has been extensively reviewed (reviewed by Rzezutka and Cook 2004). As previously mentioned, the most relevant factors affecting virus survival in the water environment are: temperature, virus association with solids, exposure to UV, and the presence of microbiota. The effect of temperature on viral persistence in water may be due to several mechanisms including protein denaturation, RNA damage, and influence on microbial or enzymatic activity (Dimmock 1967; Melnick and Gerba 1980; Deng and Cliver 1995). Early studies pointed to damage to virion proteins as the primary target for viral inactivation at high

temperatures, although damage to both protein and RNA occurs at all temperatures (Dimmock 1967). Even though all viruses persist better at lower temperatures than at higher temperatures, some enteric viruses, such as HAV and parvovirus, do exhibit higher thermal resistance than other viruses.

As mentioned earlier in this chapter, virus adsorption to particulate material increases the persistence of enteric viruses in the water environment (Gerba and Schaiberger 1975; La Belle et al. 1980; Rao et al. 1984; Sobsey et al. 1988), although differences have been observed among study locations (La Belle et al. 1980; Sano et al. 2011; Pérez-Sautu et al. 2012). The increased virus survival in the presence of sediment has important implications in the marine environment, because fecal contamination of coastal areas results in contamination of shellfish harvesting areas, accumulation of solid-associated viruses in sediments with sediments acting as virus reservoirs, and finally accumulation of viruses in shellfish. Additionally, virus uptake by molluskan bivalves is enhanced by the presence of particulate material (Landry et al. 1983).

Although self-purification processes are reported to be more pronounced in seawater than in river water (Matossian and Garabedian 1967; Gironés et al. 1989), the effect of salinity on virus stability is variable. Thus, many studies have reported enhanced removal of virus infectivity in saline solution compared with distilled water (Dimmock 1967; Salo and Cliver 1976), whereas others report no significant effect of salinity on virus persistence (Lo et al. 1976; Fujioka et al. 1980). In any case, the self-depuration capacity of water is finite.

Several observations demonstrate the potential involvement of native aquatic microorganisms in the inactivation of viruses, particularly in marine habitats. However, data on the successful isolation of microorganisms with virucidal properties are scarce (Fujioka et al. 1980; Gironés et al. 1990; Bosch et al. 1993). Additionally, the ability of bacteria to inactivate viruses is usually lost while subculturing the microorganisms in the laboratory (Gunderson et al. 1968; Katzenelson 1978), although in a few studies, such bacteria could be subcultured for more than 1 year without losing their antiviral activity (Gironés et al. 1990; Bosch et al. 1993). In some studies, the virucidal agents in the tested waters could not be separated from the microorganisms (Shuval et al. 1971; Denis et al. 1977; Fujioka et al. 1980; Ward et al. 1986; Gironés et al. 1990), whereas in others the virucidal activity could be separated from the bacteria (Matossian and Garabedian 1967; O'Brien and Newman 1977; Toranzo et al. 1983; Bosch et al. 1993). The antiviral activity seems to be based on proteolytic bacterial enzymes that inactivate virus particles in water by cleavage of viral proteins, thus exposing the viral RNA to nuclease digestion (Toranzo et al. 1983; Gironés et al. 1990; Bosch et al. 1993).

It seems reasonable to assume that environmental factors and the compositional makeup of a given type of water may be substantially different from one geographical location to another, which implies that different data of virus persistence are produced when the same viral strain is suspended in water sampled from different sites (Bosch et al. 1993). Furthermore, it is highly likely that natural waters, particularly in the marine environment, contain a variety

of potential antiviral factors, and that the antiviral action observed is generally the expression of the most dominant factor(s) present in any given water source.

3.2. Virus Persistence in Soil

Diseases associated with soil have been categorized according to the origin of the etiological agent as follows (Weissman et al. 1976; Santamaría and Toranzos 2003): (a) soil-associated diseases that are caused by opportunistic or emerging pathogens belonging to the normal soil microbiota; (b) soil related diseases that result in intoxication from the ingestion of food contaminated with entero- or neurotoxins; (c) soil-based diseases caused by pathogens indigenous to soil; and (d) soil-borne diseases caused by enteric pathogens that get into soil by means of human or animal excreta. In this latter category are included viruses transmitted through the fecal-oral route.

The transport of viruses through soil to groundwater and then to the community has been a topic of great concern. Many epidemics of infectious diseases have been attributed to the consumption of contaminated groundwater, casting soil as a vector and source of important human disease agents (Asano and Cotruvo 2004; Craun et al. 2010). There is a concern about a possible increase in soil-borne diseases in human population, given the land disposal practices of sewage and sewage sludge. In developing countries, untreated domestic wastewater is used in agricultural irrigation, presenting a high risk to farm workers and to consumers of food products irrigated with wastewater (Strauss 1994). In spite of the clear public health implications of the occurrence and survival of viruses in the soil compartment, studies on the fate of viruses in soil are scarce due to the complexity of the methodologies for virus extraction from soil.

The most relevant factors controlling virus transport through soil are soil type, water saturation state, pH, conductivity of the percolating water, and soluble organic matter (Table 13.2). The type of soil has a great influence on the level of viral transport. Fine-textured soils tend to absorb viruses more readily than coarsely textured soils. As a general rule, sandy soils are relatively poor adsorbents of enteric viruses, whereas soils with clay content of 30–100 % are excellent adsorbents (Sobsey et al. 1980). In consequence, viral adsorption increases with increasing clay mineral content (Gerba et al. 1981). The high adsorptive properties of a clay soil will prevent virus transport to another matrix, such as groundwater, whereas coarse soil will not.

Microbial movement in soils is also greatly dependent on the water saturation state. When the soil is saturated, all pores are filled with water, which allows faster virus transport through the soil because virus contact with the soil has been diminished. When the flow is unsaturated, the viruses are in closer contact with the soil, thus promoting virus adsorption to the soil (Santamaría and Toranzos 2003). Goyal and Gerba (1979) considered soil pH as the single most important factor influencing viral adsorption, although the combined effect of organic matter and clay content, and cation-exchange capacity, could

surpass the sole soil pH effect. At ambient conditions, viruses are usually negatively charged, thus being attracted to and entrapped by positively charged material in soil (Sobsey et al. 1980). In neutral and alkaline soil situations, viruses will not bind to any particulate matter and will be allowed to move freely in soil. There are, however, many exceptions to these general rules. Virus absorption to soil is also affected by cation concentrations. Cations favor virus adsorption to soil by reducing their repulsive forces. Sewage wastes provide an environment that enhances virus retention to soil, while this retention would be low in distilled water. As a matter of fact, distilled water may actually lead to the elution of viruses from soils, favoring virus mobilization and transport through soil. On the other hand, soluble organic matter will compete with the virus for soil adsorption sites. Likewise, humic and fulvic acids will also compete with the virus and will reduce the level of adsorption of viruses to the soil (Sobsey and Hickey 1985). Wei et al. (2010) investigated murine norovirus (MNV) and HAV stability on three types of differently treated biosolids at 20 and 4 °C and they reported that both viruses were inactivated rapidly in alkaline pH biosolids.

3.3. Virus Persistence in Aerosols

Aerosols are an important means of virus transmission in humans. Various authors have reported the isolation of enteric viruses from aerosols produced by sludge-treatment plants (Fannin et al. 1985; Fattal and Shuval 1989; Pfirrmann and Bossche 1994; Alvarez et al. 1995; Carducci et al. 1999). The presence of microorganisms in aerosols generated from wastewater-treatment processes or in treated wastewater for agricultural irrigation is a potential danger to human health (Teltsch et al. 1980; Alvarez et al. 1995). In hospitals, aerosolization of vomit was reported to be of major importance in the transmission of NoV infection during outbreaks, while cleaning vomit or feces from patients did not significantly increase the risk of developing gastroenteritis (Chadwick and McCann 1994). Members of the *Caliciviridae* family have been reported to be fairly stable in aerosols (Donaldson and Ferris 1976). The most important factors affecting the stability of viruses in the aerosol state are temperature, pH, relative humidity, moisture content, size of the aerosol particle, composition of the suspending medium, sunlight exposure, air quality, and virus type.

The basis of virus inactivation in aerosols is poorly understood, although mechanisms for bacteriophage inactivation in aerosols have been proposed (Trouwborst et al. 1974). At high relative humidity, surface alteration of the virion has been reported, whereas at low relative humidity virus inactivation appears to be mediated by the removal of structural water molecules. Relative humidity seems to confer a protective effect on aerosolized non-enveloped virus particles. Thus, poliovirus was more stable in aerosol at 22 °C at high relative humidity than at low relative humidity (Harper 1961). Picornavirus infectious RNA may be detected at all humidity levels, suggesting that virus inactivation is caused by virion capsid damage (Akers and Hatch 1968). High

relative humidity and low temperature enhance the persistence of bovine rotavirus in aerosols (Moe and Harper 1983; Ijaz et al. 1985), although simian rotavirus SA11 survival in aerosols seems to be the best at intermediate relative humidity levels (Sattar et al. 1984). In any case, human, simian, and calf rotavirus strains may be detected in aerosols after as long as 10 days (Moe and Harper 1983; Sattar et al. 1984; Ijaz et al. 1985), although discrepancies, probably due to methodological differences, are found among these studies. Aerosolized adenovirus particles also show increased persistence at high relative humidity and low temperature (Miller and Artenstein 1967; Elazhary and Derbyshire 1979).

Contrarily to non-enveloped viruses, viruses with an outer lipid envelope seem to be more stable at lower relative humidity (Hemmes et al. 1960). After 6 days at 20 °C and 50 % relative humidity, infectious human coronavirus particles could be recovered in aerosols (Ijaz et al. 1985). Virus infectivity in aerosols is also affected by solutes in the suspending media used for aerosolization. Addition of salts and proteins in the suspending media provides a protective effect against dehydration and thermal inactivation of aerosolized picornaviruses (McGeady et al. 1979; Reagan et al. 1981) and may also influence the rehydration rate during sample re-humidification prior to the infectivity assay (Benbough 1969).

3.4. Virus Persistence on Fomites

Outbreaks of acute gastroenteritis and hepatitis are a matter of concern in institutions such as, hospitals, daycare centers, nurseries, schools, restaurants, and military quarters. Many of these outbreaks have been suspected to be caused by vehicular transmission of agents through contaminated environmental surfaces (fomites). Stools from patients with diarrhea or hepatitis contain a very high number of the causative virus, and a single vomiting episode of an individual suffering from NoV gastroenteritis may expel 3×10^7 virus particles, all of which are able to contaminate fomites (Cheesbrough et al. 1997; Green et al. 1998).

It has been demonstrated that human enteric viruses are able to survive on several types of materials commonly found in institutions and domestic environments long enough to represent a source for secondary transmission of disease (Hendley et al. 1973; Sattar et al. 1986, 1987; Ansari et al. 1988; Mbithi et al. 1991; Abad et al. 1994, 2001). The stability of health-significant human enteric viruses has been investigated on various non-porous (aluminum, china, glazed tile, glass, latex, plastic, polystyrene and stainless steel) and porous (cloth, different types of papers and cotton cloth) surfaces (Sattar et al. 1986; Abad et al. 1994, 2001; Boone and Gerba 2007). As a general conclusion, when dried on environmental fomites, HAV and rotavirus are more resistant to inactivation than enteric adenovirus, astrovirus, and poliovirus.

The higher stability of HAV in comparison with poliovirus, both of which belong to the *Picornaviridae* family, is due to the inherently more stable

molecular structure of HAV capsid, concordant with the special codon usage described for this virus (Sánchez et al. 2003). In fact, it appears undeniable that poliovirus, which has been extensively employed as a model to elucidate enteric virus behavior in many scenarios, may fail to provide an adequate indication of the persistence of other human enteric viruses, such as HAV, astrovirus, or rotavirus, dried on fomites (Sobsey et al. 1988; Mbithi et al. 1991; Abad et al. 1994, 2001).

The resistance to desiccation appears to be of major significance in determining the ability of a virus strain to survive on fomites. A pronounced loss in virus titer at this stage dramatically reduces the chances of subsequent virus persistence. On the contrary, viruses involved in outbreaks probably transmitted through faecally contaminated environmental surfaces (i.e., HAV, NoV, rotavirus, or astrovirus) show little decay at the desiccation step (Mahl and Sadler 1975; Keswick et al. 1983; Sattar et al. 1986; Sobsey et al. 1988; Abad et al. 1994, 2001).

In spite of the experimental data on virus persistence on environmental surfaces, it is generally very difficult to determine whether, and to what extent, fomites play a role in the spread of infectious agents. Keswick et al. (1983) have suggested that the prevalence of asymptomatic infections in daycare facilities may make contaminated surfaces in these environments a reservoir of infection for previously uninfected inmate children and their family contacts.

Because the fecal-oral route is the common means of enteric virus transmission, it seems reasonable to evaluate the effect of fecal material on the persistence of virus on fecally contaminated fomites. Again, data on the protective effect of feces on viruses are contradictory; fecal matter appears to affect the survival of enteric viruses in opposite ways, depending on the type of surface and the virus strain (Keswick et al. 1983; Sobsey et al. 1988; Abad et al. 1994).

Survival of NoV on fomites has been investigated by using surrogates or using molecular techniques. Studies using NoV surrogates are more abundant. Clay et al. (2006) investigated FCV survival on computer mouse, keyboard keys, telephone wire, telephone receiver, telephone buttons, and brass disks representing faucets and door handle surfaces. This study concluded that survival of FCV varied with fomite type. FCV was still infectious for up to 3 days on telephone buttons and receivers, for 1 or 2 days on computer mouse, and for 8–12 h on keyboard keys and brass. Mattison et al. (2007) also used FCV to investigated NoV survival on stainless steel. Temperature substantially affects the survival of FCV, which is able to persist for long periods of time dried onto glass coverslips with log reductions of 4.75 after 2 months and 3 weeks, at 4 °C and room temperature, respectively (Doultree et al. 1999). The authors suggested that the effect of temperature on FCV stability may reflect the greater prevalence of NoV infections in cooler seasons (Lopman et al. 2003).

Cannon et al. (2006) reported long-term persistence of FCV and MNV suspended in a fecal matrix and inoculated onto stainless steel coupons at 4 °C, but at room temperature MNV was more stable than FCV. Recently, MNV

was used as a NoV surrogate to investigate survival on food contact surfaces. MNV infectivity on stainless steel rapidly decreased by more than 2 log, and a complete loss of infectivity was reported at day 30 (Takahashi et al. 2011). Additionally, they also showed that the presence of food residues increased the survival of MNV, whereas only 1.4 log reduction of infectivity was reported at day 30.

D'Souza et al. (2006) investigated the stability of NoV, NoV RNA and FCV on stainless steel, formica and ceramic coupons. NoV and FCV were detected on all 3 surfaces up to 7 days post inoculation. NoV RNA was not detected beyond 24 h on stainless steel. Moreover, in this study, stainless steel coupons were inoculated with NoV or FCV and allowed to dry after which lettuce leaves were exposed to the surface of the coupons at various contact pressures. Results showed that transfer of both NoV and FCV from stainless steel surfaces to lettuce occurred easily. Recently Lopez et al. (2013) examined the effect of low and high relative humidity on fomite-to-finger transfer efficiency of poliovirus from several common fomites, showing that transfer efficiencies were greater under high relative humidity for both porous and nonporous surfaces. Gloves also may serve as a source of virus second transmission, enteric viruses could be transferred in an infectious state from gloves to other surfaces or food and vice versa (Verhaelen et al. 2013).

3.5. Virus Persistence on Hands

Strong evidence indicates that virus-contaminated hands play a major role in the spread of enteric viruses, particularly in institutional settings and during food preparation.

Contaminated human hands can transfer the virus to inanimate objects or food products, which may then spread the virus to susceptible persons (Hendley et al. 1973; Ansari et al. 1988; Mbithi et al. 1992; Bidawid et al. 2001a; Tuladhar et al. 2013). It was ascertained in these studies that rotavirus and HAV could retain infectivity for several hours on skin and could be transferred in an infectious state from fingertips to other surfaces and vice versa. For norovirus, MNV infectivity transfer from finger pads to stainless steel ranged from $13 \pm 16\,\%$, whereas similar results were found for NoV GI.4 and GII.4 transfers measured in PCR units.

Enteric virus transfer between hands was apparently influenced by moisture. Moisture would mediate suspension of virus particles and facilitate their movement between touching surfaces; drying would reduce this effect. Laboratory studies have shown that viruses persist better in the environment at high relative humidity and at low temperatures (Sattar et al. 1984; Sobsey et al. 1988; Abad et al. 1994; Bidawid et al. 2001a). However, as mentioned above, data on the effect of relative humidity on enteric virus survival is contradictory. These reported differences, particularly affecting rotavirus persistence, are difficult to explain but may be due to differences in the methodologies employed in these studies.

4. STABILITY OF ENTERIC VIRUS IN FOOD PRODUCTS

The most important factors affecting the stability of viruses in food products are virus type, temperature, pH, relative humidity, moisture content, sunlight exposure and type of food. This latter factor may have a great impact depending on the type of surface, for instance the presence of crevices and hair-like projections in berries may shield the viruses against environmental modifications or the presence of natural antiviral compounds in the food itself.

4.1. Stability of Enteric Viruses on Chilled Products

In minimally processed fruits and vegetables, chilled storage temperatures (2–11 °C) typically retard respiration, senescence, product browning, moisture loss, and microbial growth, but may contribute to the survival and transmission of enteric viruses (Seymour and Appleton 2001; Rzezutka and Cook 2004). A variety of enteric viruses have been examined for the effects of chilled temperature on their survival in a range of food matrices (reviewed by Baert et al. 2009) (Table 13.3). Most of the studies found that enteric viruses remained infectious for periods exceeding the shelf-life of products (Table 13.3). On vegetables, Croci et al. (2002) evaluated HAV survival on lettuce, carrots and fennel, reporting complete inactivation of HAV by day 4 and 7 for carrots and fennel, respectively. On lettuce a slight decrease was observed over time. Sun et al. (2012) recently reported that HAV survived more than 20 days during storage at 3–10 °C on surface inoculated green onions. Shieh et al. (2009) investigated the survival of HAV on fresh spinach leaves in moisture- and gas-permeable packages that were stored at 5.4 °C for up to 42 days, reporting only a 1 log reduction of HAV infectivity over 4 weeks of storage. In shellfish, HAV inoculated in commercially prepared marinated mussels showed a 1.7 log reduction of infectivity after 4 weeks of storage at 4 °C (Hewitt and Greening 2004).

The stability of HAV and PV inoculated in bottled water was studied at 4 °C (Biziagos et al. 1988). Infectious HAV and PV were detected after 1 year of storage, with less than 1 log reduction. This study also reported that HAV stability was dependent on the proteinaceous concentration added to the water.

Attempts to evaluate stability of human NoV in food products have been performed by using molecular techniques alone or combined with pretreatments to assess infectivity. Mormann et al. (2010) reported no reductions on NoV titers after RNase pretreatment during cooling for lettuce (5 days, 11 °C), apples (7 days, 11 °C) and mincemeat (2 days, 6 °C). Lamhoujeb et al. (2008) demonstrated that NoV survived for at least 10 days on refrigerated lettuce and turkey. In mussels no reduction on NoV titers were reported after 4 weeks of storage at 4 °C (Hewitt and Greening 2004).

Several studies have also estimated NoV stability by using surrogates. Mattison et al. (2007) investigated the survival of FCV on lettuce and straw-

Table 13.3 Survival of NoV and HAV in artificially inoculated foodstuffs during refrigeration storage

	Virus	*Food product*	*Process*	*Log reduction*	*Reference*
Refrigeration	NoV	Apple	7 days at 11 °C	0.2 (±0.76)	Mormann et al. (2010)
		Lettuce	5 days at 11 °C	0.1 (±0.15)	Mormann et al. (2010)
			10 days at 7 °C	1.78	Lamhoujeb et al. (2008)
		Mincemeat	2 days at 6 °C	0.0 (±0.61)	Mormann et al. (2010)
		Marinated mussels (pH 3.75)	28 days at 4 °C	0.0	Hewitt and Greening (2004)
		Raspberries	7 days at 4 °C	0.0	Verhaelen et al. (2012)
			7 days at 10 °C	0.3	
		Strawberries	7 days at 4 °C	0.0	Verhaelen et al. (2012)
			7 days at 10 °C	0.3	
		Turkey	10 days at 7 °C	0.0	Lamhoujeb et al. (2008)
	HAV	Bottled water	360 days at 4 °C	0.68	Biziagos et al. (1988)
		Carrot	4 days at 6 °C	Complete inactivation	Croci et al. (2002)
		Fennel	7 days at 6 °C	Complete inactivation	Croci et al. (2002)
		Green onions	29 days at 3 °C	1.17 (±0.10)	Sun et al. (2012)
			16 days at 10 °C	1.15 (±0.15)	
		Lettuce	9 days at 6 °C	2.46 (±0.17)	Croci et al. (2002)
		Spinach	28 days at 5.4 °C	1	Shieh et al. (2009)
		Marinated mussels (pH 3.75)	28 days at 4 °C	1.7	Hewitt and Greening (2004)

berry mimicking the contamination of produce by food handler disks. Approximately a 2 log reduction was observed on lettuce after 7 days at 4 °C while a 2.5 log reduction was observed on strawberries after 6 days at 4 °C. MNV, NoV GI and GII showed greater viral persistence on raspberries as compared to strawberries, especially at 21 °C (Verhaelen et al. 2012).

Porcine sapovirus (SaV) is a culturable enteropathogenic calicivirus and it has recently used as norovirus surrogate to examine virus attachment to lettuce. Recently, Wang et al. (2012) showed that SaV remained infectious on lettuce after 1 week of storage at 4 °C. For other enteric viruses, Kurdziel et al. 2001 estimated the D-values (number of days after which the initial virus numbers had declined by 90 %) for poliovirus in various vegetables. The resulting D-values were 11.6 days for lettuce, 14.2 days for white cabbage, and no decline for green onion and fresh raspberries for 2 weeks. The survival of poliovirus was investigated in commercial yogurt, reporting infectious viruses after 24 days of storage at 4 °C (Strazynski et al. 2002).

Rotavirus SA-11 survived on lettuce, radish, and carrots for 25–30 days at 4 °C (Badawy et al. 1985) and coronavirus remained infectious for at least 14 days on lettuce surfaces under household refrigeration conditions (Mullis et al. 2012).

In general, the above-mentioned studies indicated that enteric viruses will survive on chilled food over the periods before deterioration of the specified food.

4.2. Stability of Enteric Viruses Under Frozen Storage

The occurrence of enteric virus outbreaks caused by to the consumption of berries and shellfish that had been frozen several months (Bosch et al. 2001; Hutin et al. 1999; Niu et al. 1992; Pintó et al. 2009; Ramsay and Upton 1989; Reid and Robinson 1987; Sanchez et al. 2002) (Table 13.1) indicates that if food is contaminated before freezing, substantial fractions of the viruses will remain infectious during frozen storage. For instance, six NoV outbreaks occurred in Europe in 2005 and involved up to 1100 people and were associated with the consumption of frozen berries imported from Poland (Falkenhorst et al. 2005; Korsager et al. 2005). The occurrence of virus outbreaks linked to imported strawberries from China, has called the attention of The European Commission. The European Commission (SANCO 12655/2012) has implemented the monitoring of NoV and HAV in some food imports in accordance with Art 15(5) of Regulation (EC) No 882/2004 (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:194:0011:0021:EN:PDF), employing the standardized CEN methodologies (ISO/TS 15216–1 and ISO/TS 15216–2; 2013).

Butot et al. (2008) extensively investigated the survival of HAV, NoV, RV and FCV, on frozen strawberries, blueberries, raspberries, parsley, and basil, concluding that frozen storage for 3 months had limited effects on HAV and RV infectivity in all tested food products, whereas in frozen raspberries and strawberries FCV infectivity showed the highest decay rate due to acid

pH. Persistence of NoV was evaluated by RT-qPCR, showing that NoV GII was less resistant than NoV GI under the tested conditions. However there was no more than 1 log difference in the reductions found for the two NoV genogroups. Likewise, after freezing of inoculated pizza product (7 and 14 days, at −18 °C) and mincemeat (8 days, −18 °C), no significant reductions in the NoV titer pretreated with RNase was observed (Mormann et al. 2010). Moreover, HAV was recently detected and typed from samples of mixed frozen berries linked to an Italian hepatitis A outbreak in 2013 (Chiapponi et al. 2014).

On shellfish, some studies have reported the presence of enteric viruses in frozen shellfish. For instance, Shieh et al. (2007) were able to detect and type HAV sequences in oysters implicated in an outbreak. These oysters were stored in the cold for 12 days and then frozen at −20 °C for 7 weeks before analysis. Sanchez et al. (2002) and Pintó et al. (2009) also detected and typed HAV from imported frozen clams that caused two outbreaks in Spain. All these results indicate that freezing has little or no effect on HAV infectivity in molluscan shellfish.

Overall, these studies showed that freezing does not ensure an adequate reduction of enteric virus if present in foods.

4.3. Effects of Relative Humidity on Enteric Virus Persistence

The influence of relative humidity on the survival of enteric viruses on different vegetables and fruits has scarcely been investigated. Stine et al. (2005) investigated the survival of HAV and FCV on lettuce, bell peppers and cantaloupe, stored at 22 °C under high (mean, 85.7–90.3 %) and low (mean, 45.1–48.4 %) relative humidity. HAV survived significantly longer than FCV, and high inactivation rates were reported under conditions of high humidity.

4.4. Stability of Enteric Viruses on Dried Food Products

Enteric virus survival in dried state has been studied mostly on inanimate surfaces or fomites, and has been reviewed earlier in this chapter. The multistate outbreak of hepatitis A associated with the consumption of sun-dried tomatoes shows that if food is contaminated before drying, substantial numbers of viruses will still remain infectious (Gallot et al. 2011; Petrignani et al. 2010).

4.5. Stability of Enteric Viruses Under Modified Atmosphere Packaging

Modified atmosphere packaging (MAP) is typically employed to slow the respiration rate of vegetables and fruits and therefore reduce the metabolism and maturation of the food products. Moreover, MAP is a way of extending the shelf life of fresh food products by inhibiting spoilage by bacteria and fungi. This technology replaces the atmospheric air inside a package with a protective gas mix. Overall this type of packaging is designed to inhibit bacterial or fungal growth and therefore is not effective against enteric viruses because they do not grow in food products. This is supported by recent reports

were the presence of enteric viruses were detected in ready-to-eat packaged leafy greens. In Canada, NoV were detected on 6 % and rotavirus in 0.4 % of lots tested from retail markets in southern Ontario. Packages with confirmed positive samples were imported into Canada (Mattison et al. 2010).

So far, only one study has evaluated the effect of various modified atmospheres on the survival of HAV on lettuce stored at room temperature and 4 °C for up to 12 days in ambient air and under various modified atmospheres (Bidawid et al. 2001a, b). The lettuce samples were stored in heat-sealed bags with the following percentages of gas mixtures (carbon dioxide [CO_2]:nitrogen): 30:70, 50:50, 70:30, and 100 % CO_2. Only at 70 % CO_2 at room temperature was a significant decline in virus survival observed. Because most commercially distributed vegetables are stored at lower CO_2 concentrations and at 4 °C, standard MAP conditions will not prevent HAV transmission.

4.6. Effects of Acidification on Enteric Virus Survival

Sauces, dressings, marinades, and similar food products depend on their acidity to prevent spoilage. They may consist of naturally acidic foods, such as tomatoes' sauce or fruit juices, or they may be formulated by combining acidic foods with other foods to achieve the desired acidity. Moreover some foods, such as vinegar and certain pickled vegetables, may develop acidity from microbial fermentation. However acidification of food is not a suitable hurdle to control enteric virus in foods since they are highly stable at an acidic pH. For instance, HAV had a high residual infectivity after 2 h of exposure to pH 1 at room temperature, remaining infectious for up to 5 h. HAV remained infectious for 90 min at pH 1 and 38 °C (Scholz et al. 1989). Similar trends are reported for human NoV, (Mormann et al. 2010) reported only a 1.7 log reduction of NoV titers pretreated with RNase after storage at 6 °C during 24 days under acid pH conditions in potato salad (pH from 5.0 to 5.5). Furthermore, no reduction in the virus titer was observed for storage in noodle salad (24 days, pH from 5.0 to 5.5) or tomato ketchup (58 days, pH 4.5). In conclusion these data show that acidification is not a suitable strategy to reduce the number of enteric viruses present on food.

5. CONCLUSIONS

Survival of enteric viruses in the environment and different food products has been well studied employing cell-adapted virus strains. However, there is a definite need for further research for the study of NoV survival. So far, NoV survival has been investigated either using surrogates or by molecular techniques. However both approaches have several drawbacks. Molecular techniques did not differentiate between infectious and non-infectious viruses while many differences have been reported between the inactivation of NoV and its surrogates, thus questioning the validity of these surrogates. Clinical

trials may be the best option, however studies with volunteers still are not accepted in many countries.

Furthermore it should be recognized, that most of the studies on virus persistence were performed under laboratory conditions and that data obtained from these studies may not truly represent their behavior under actual field conditions. For instance, several studies have reported virus intake by vegetables, although almost all data on virus persistence on vegetables have been obtained by surface inoculation of the target virus.

Overall, data provided in this review shows that enteric viruses are very stable in the environment and in food products. As a consequence, emphasis should be on prevention of contamination by implementing good hygienic, agricultural, and manufacturing practices. Strategies to reduce the risk of foodborne outbreaks of enteric viruses should focus on preventing foods from becoming contaminated. In developing countries, young children should be kept away from areas where fresh produce is grown and harvested. This measure is important for hepatitis A infection, since in developing countries this disease is usually acquired during early childhood as an asymptomatic or mild infection. Education of workers, with an emphasis on hygiene; providing facilities for maintaining cleanliness; and the use of treated water in production and processing will be major deterrents to contamination of food with enteric viruses. Shellfish harvesting areas should be monitored for NoV and HAV contamination. Moreover, because enteric viruses are easily transferred from utensils/fomites to food/persons and vice versa, efforts have to be taken to prevent cross-contamination in the different scenarios.

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