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Natural persistence of food- and waterborne viruses

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Abstract: This chapter summarises data on the persistence of food- and waterborne viruses in the natural environment and discusses the different factors which can affect this persistence. Conventional and alternative methods by which persistence can be studied are described, and the natural factors influencing virus persistence outside the host organism are discussed. Available data concerning virus persistence in water, soil, on surfaces and in food products are reviewed.

Key words: food and waterborne viruses, methods for study of virus persistence, factors affecting virus persistence, water, soil, surfaces, food products.

9.1   Introduction

Data on the persistence of food- and waterborne viruses in the environment outside the host organism are very important to the understanding of viral ecology and thus in determining the risk to the human population represented by these viruses. This chapter is focused mainly on the existing data regarding virus persistence in the most important matrices associated with the spread of food- and waterborne viruses (i.e., water, soil, food-related surfaces and food products). As virus persistence outside the host organism is affected by a combination of biological (presence of envelope, type of virus genome and presence of other micro-organisms), physical (temperature, relative humidity and UV) and chemical (pH, presence of salts and adsorption state) factors, the chapter contains a special section which reviews these factors and their general influence on virus survival.

To obtain information about the presence and persistence of viruses in the environment, appropriate methods are needed; that is, methods which are able to distinguish between infectious and non-infectious particles and are thus suitable for use to determine the real risk of infection. Therefore, common (cell culture and polymerase chain reactions) and new alternative methods (e.g., a special pre-treatment of samples) available for such studies are also discussed. Virus transmission via food and the environment is now a well-recognised problem. This awareness of foodborne and waterborne viruses emphasises the need for data regarding the persistence of viruses in the environment and the effects of preservation methods upon viruses. During recent years, there has been an increase in the number of survival and inactivation studies. Despite this, there is still a lack of general information about the risk to the human population represented by these viruses, which remains to be fully determined. The important foodborne and waterborne viruses (e.g. norovirus, hepatitis E virus and several strains of hepatitis A virus) cannot be commonly cultivated in the laboratory, which hinders the study of their stability in food and in the water environment. To address this problem, cultivable surrogate viruses, which are genetically related to the strains which infect humans, are used as substitutes for these viruses in order to obtain preliminary information about their behaviour in the environment. However, the use of such surrogates is questionable due to the differences in susceptibility to environmental factors even between different surrogates. At present, novel cultivation methods and approaches based on molecular methods are being developed. Although these methods are promising, none are commonly applicable to all viruses and thus further development is needed. In addition, the study of virus persistence in the environment would benefit greatly from the standardisation of experimental protocols, which would allow the generation of complete and comparable data about the natural persistence of viruses. Therefore, standardised methods applicable for the detection of infectious viral particles in different kinds of matrices and for the subsequent statistical analysis of results need to be developed.

Environmental and especially food virology are relatively new subject areas. To date, most experiments dealing with virus persistence in the environment have been conducted at the national level. At the international level, only a limited number of projects focused on waterborne and foodborne viruses have been implemented: for example, Virobathe (http://www.virobathe.org) which evaluated methods for detecting noroviruses and adenoviruses in recreational waters; VITAL (Integrated Monitoring and Control of Foodborne Viruses in European Food Supply Chains; http://eurovital.csl.gov.uk) which focused on foodborne viruses within selected food supply chains from farm to market; and COST 929 ENVIRONET (http://www.cost929-environet.org/index.html) as an international network for environmental and food virology. These projects have contributed to our understanding of foodborne and waterborne viruses and enabled the risks associated with the transmission of these viruses to be partially determined. Notably, the research group CEN/

WG6/TC275/TAG4 'Detection of viruses in foods' has been established to investigate the occurrence of foodborne viruses and introduce standard methods for their detection. Current information about virus persistence and appropriate methodologies is available in the form of research articles and several reviews; for example, data concerning viral persistence, particularly in fresh food, were summarised by Seymour and Appleton (2001) and Rzezutka and Cook (2004); overviews of data with respect to reduction/inactivation of viruses by food preservation methods were published by Baert *et al*. (2009) and Hirneisen *et al*. (2010) and methods for assessing the infectivity of enteric viruses in environmental samples were discussed by Rodriguez *et al*. (2009).

9.2 Methods for studying persistence

Studies of virus persistence and the determination of a virus' ability to retain infectivity are usually performed according to basic principles. Generally, tested samples are artificially contaminated by a virus suspension containing a determined concentration of infectious particles, and the samples are then stored or processed under defined conditions. Subsequently, the viruses are extracted from the sample and the infectious units are quantified. The comparison between the number of infectious viruses isolated from a tested sample and the number which was originally introduced provides data about the persistence of virus infectivity (Rzezutka and Cook, 2004).

Studies can be performed under laboratory or under natural conditions; both approaches have their advantages and disadvantages. The advantages of studies carried out under laboratory conditions are the possibilities for precisely defined and stable conditions (e.g., temperature, relative humidity and pH). Virus persistence is influenced by combinations of biological, physical and chemical factors, the various permutations of which it is not feasible to successfully recreate under laboratory conditions. Thus, laboratory experiments can be used only to define the effect of individual factors on virus persistence. In contrast, experiments performed under natural conditions provide compact data about virus persistence in the environment. However, problems could arise with regard to the definition of specific conditions influencing virus persistence.

Successful detection of infectious viral particles in the environment is complicated by several factors, such as virus size; the wide variation among and within viral genera; low concentration; the presence of substances which can interfere with analysis procedures; the limits of detection of different techniques; and the absence of reliable controls (Vasickova *et al*., 2010). Various methods have been developed for the isolation of infectious viral particles from different kinds of matrices (e.g., water, soil, food and surfaces). Their basic principles and aims are similar; they involve the separation of viral particles (elution, washing or filtration) from the sample and their subsequent

concentration to an amount which is suitable for proper detection. Successful detection depends on both the extraction method and the detection techniques. The standard methods for the detection of infectious viruses and thus viral survival under different conditions still involve cell culture. Susceptible cell lines in which the viruses propagate are required. The advantages are the direct detection of infectious viral particles and their sensitivity; theoretically, these methods can detect a single viable viral particle (Reynolds *et al.*, 2001). The quantification of infectious virions can be achieved through the use of cell culture in a quantitative format, for example, plaque assay. Routinely cultivable viruses include poliovirus and related viruses from the family *Picornaviridae*, astroviruses, rotaviruses, and cell culture-adapted strains of hepatitis A virus (HAV) (Richards, 2012). However, each virus type or even strain has different capabilities and thus may require different conditions for effective propagation in cell culture; for example, not all enteroviruses are able to propagate on one cell line (Dahling, 1991). Cell culture methods can also be time-consuming: the time required varying between 4 and 30 days depending on the virus. Furthermore, detection is problematic in the case of those viruses which cannot be grown in conventional cell culture: for example, human norovirus (NoV) and hepatitis E virus (HEV) (Rodriguez *et al.*, 2009; Vasickova *et al.*, 2010). Although NoVs have been reported growing in highly differentiated 3D cell cultures (Straub *et al.*, 2007), these systems require specialised equipment and extensive experience, and have proven difficult to successfully reproduce (Parshionikar *et al.*, 2010).

The lack of a suitable cell culture method for the detection of infectious particles of non-cultivable viruses such as HAV or NoV has led to the use of surrogate viruses, which provides at least predictive data about the survival of non-cultivable viruses in the environment. The selection of a proper surrogate virus is usually based on its ability to propagate in cell cultures, and its genetic, biological, physical and chemical relatedness to the virus which is to be isolated. Although several surrogates have been used specifically for NoV (Table 9.1; Richards, 2012), differences have been found between the inactivation of NoV and these viruses. It was also shown that the susceptibility of different NoV surrogates to temperature, environmental and food-processing conditions or disinfectants differs dramatically between these viruses (Cannon *et al.*, 2006). Moreover, several studies reported that HAV strains that have adapted to cell culture have diverse sensitivities to heat and high pressure (Shimasaki *et al.*, 2009). Differing modes of inactivation can be anticipated also among other kinds of viruses. However, differences between strains of viruses belonging to the same genus may not be as pronounced as those between the non-cultivable viruses and their surrogates. Therefore, data obtained by the use of surrogate viruses should be evaluated and presented carefully as presumptive evidence of how pathogens may respond to different treatments. Thus, the use of surrogate viruses and extrapolation from the persistence of surrogates to the persistence of non-cultivable viruses is questionable (Richards, 2012).

Table 9.1 Summary of surrogate viruses and their characteristic

Surrogate virus	Classification (family/genus)	Genome	Host	Infection	Represented virus	References
Feline calicivirus (FCV)	*Caliciviridae/Vesivirus*	+ssRNA	Cat	Respiratory infection	Norovirus	Duizer *et al.*, 2004
Canine calicivirus	*Caliciviridae/Vesivirus*	+ssRNA	Dog	Glossitis, enteritis	Norovirus	Duizer *et al.*, 2004
Murine Norovirus (MNV)	*Caliciviridae/Norovirus*	+ssRNA	Mouse	Enteritis	Norovirus	Takahashi *et al.*, 2011
Attenuated hepatitis A virus (HAV)	*Picornaviridae/Hepatovirus*	+ssRNA	Human		Hepatitis A virus	Hewitt and Greening, 2004; Kingsley *et al.*, 2005
Attenuated poliovirus	*Picornaviridae/Enterovirus*	+ssRNA	Human		Poliovirus	Alvarez *et al.*, 2000
Rotavirus SA-11	*Reoviridae/Rotavirus*	dsRNA	Monkeys		Rotavirus	Kingsley *et al.*, 2005; Raphael *et al.*, 1985
Phage MS2	*Leviviridae/Levivirus*	+ssRNA	*Escherichia coli*		Norovirus, HAV, enterovirus, rotavirus	Dawson *et al.*, 2005; Helmi *et al.*, 2008
Phage ΦX174	*Microviridae/Microvirus*	ssDNA	*Escherichia coli*		Norovirus, HAV, enterovirus, rotavirus	Helm *et al.*, 2008; Dawson *et al.*, 2005
Virus-like particles					Norovirus, rotavirus	Kingsley *et al.*, 2005; Caballero *et al.*, 2004

ss single-stranded; ds double-stranded; + positive sense.
Source: Supplemented and adapted from Baert *et al.* (2009).

Molecular techniques are an alternative method, particularly for the detection of viral genomes. These methods, based on polymerase chain reaction (PCR) (Malorny *et al.*, 2003; Shimasaki *et al.*, 2009), nucleic acid sequence-based amplification (NASBA) (Cook, 2003; Casper *et al.*, 2005) or their quantitative format (qPCR, qNASBA), represent highly sensitive and specific assays. Molecular techniques can be used for all types of viruses (in the case of RNA viruses it is necessary to run a reverse transcription reaction prior to PCR), can determine the presence of different agents in the same sample, allow the identification of non-cultivable viruses, are rapid and can be used to quantify the viral load in the sample. Besides, additional sequencing of the amplicons allows the establishment of epidemiologic associations. The disadvantage of molecular methods is that, when used alone, they are not able to distinguish between infectious and non-infectious viruses. This is due to the ability of the viral capsid to protect nucleic acid even in non-infectious particles in many instances (Ogorzaly *et al.*, 2010). Therefore, the use of PCR or NASBA has limited application for persistence studies.

In the case of commonly cultivable viruses, the integration of cell culture and PCR (i.e., integrated cell culture-polymerase chain reaction; ICC-PCR) can allow the detection of infectious viruses in the space of hours or days compared with the days or weeks necessary with cell culture alone (Reynolds *et al.*, 2001; Gallagher and Margolin, 2007). The detection of enteroviruses in water samples, for example, is reduced to 5 days. The assay is based on an initial replication of viral particles using an appropriate cell culture for a short period, followed by PCR amplification of a specific part of the viral genome. The sensitivity of this method is comparable to that obtained using a second passage of cell culture (Rodriguez *et al.*, 2009). So far the use of ICC-PCR has been described for the detection of enteroviruses, HAV, enteric adenoviruses, reovirus and astroviruses (Shoeib *et al.*, 2009; Rigotto *et al.*, 2010; Schlindwein *et al.*, 2010). Despite the major advantages of this method, namely that it overcomes the limitations of cell culture and PCR methodologies when used alone, a system capable of detecting infectious non-cultivable viruses is still lacking.

To address the limitations of PCR-based methods several new approaches have been developed recently (Gilpatrick *et al.*, 2000; Nuanualsuwan and Cliver, 2002; Parshionikar *et al.*, 2010; Li *et al.*, 2011). Generally, these include additional sample pre-treatment steps prior to nucleic acid isolation or PCR. These steps utilize the essential properties of the viral capsid, which are associated with the loss of infectivity of viruses (Cliver, 2009a). However, these methods have been successfully used to prevent the transcription and amplification only of certain kinds of inactivated viruses, and serious limitations have arisen during their application (Table 9.2). Despite this, with further development these assays have the potential to provide more information about virus persistence.

Generally, for public health protection, the usefulness of a method is determined by its applicability to all cultivable and non-cultivable strains

Table 9.2 Summary of pre-treatment approaches prior to PCR to distinguish between infectious and non-infectious viruses

Pre-treatment prior to PCR	Rationale	Advantages	Disadvantages	References
Proteinase and RNase treatment	The capsid of infectious particles is resistant to proteinase digestion and thus protects the genome against RNases.	Usable for non-cultivable viruses Practical and easy pre-treatment	Works only in defined conditions (inactivation at 72°C or by hypochlorite)	Nuanualsuwan and Cliver, 2002; Nuanualsuwan and Cliver, 2003
Antibody capture of the virus	The capsid of inactivated virus might alter its antigenic properties (changes in protein conformation) thus resulting in the virus losing the ability of being recognised by specific antibodies.	Isolation of infectious viruses from large volume samples (e.g., water) Usable for non-cultivable viruses	Detection depends on antigenic properties of viral capsid Effective only if the antigenic properties of the viral capsid are defined	Gilpatrick et al., 2000; Schwab et al., 1996
Attachment of virus to cell monolayer	The capsid of inactivated viruses changes its antigenic properties (changes in protein conformation) and thus the virus is unable to attach to receptors of the monolayer.	Able to distinguish between infectious and non-infectious viruses (in defined conditions)	Applicable only for cell-cultivable viruses	Li et al., 2011; Nuanualsuwan and Cliver, 2003
Intercalating dyes treatment (Propidium monoazid; PMA)	The PMA penetrates the damaged or compromised capsid of the inactivated virus and binds covalently to the genome upon exposure to visible light, which prevents detection of the genome using PCR.	Usable for non-cultivable viruses	Works only in defined conditions (inactivation at 72 and 37°C or by hypochlorite)	Fittipaldi et al., 2010; Parshionikar et al., 2010

of viruses, its detection of viral infectivity and the rapidity with which the results are obtained (Parshionikar *et al.*, 2010). A combination of molecular techniques and cell culture methods should be used (Cliver, 2009b), but this does not solve the problem of non-cultivable viruses such as NoV and HEV. The ideal solution would involve a single and simple pre-treatment of any kind of sample that would quickly preclude nucleic acid amplification from non-infectious viruses (Cliver, 2009b). None of the pre-treatment procedures is applicable for all viruses and further optimization is needed.

9.3 General factors affecting the natural persistence of viruses

The potential for viral spread depends in large part on the ability of viruses to persist in the environment (Boone and Gerba, 2007). The infectious viral particles of non-enveloped viruses consist of two major components: the viral genome and the genome-protecting capsid. Infectivity requires the functional integrity of both of these components; that is, infection will occur only if the viral genome (either DNA or RNA) has retained its functional integrity and if the undamaged capsid is able to attach to the host cell receptor and initiate the process by which the nucleic acid enters the host cell (Cliver, 2009a). Many viruses have an envelope that covers the capsid and helps viruses to enter the host cell. Thus, the persistence (preservation of infectivity) of enveloped viruses is also strongly dependent on the integrity of the envelope. Viruses cannot replicate outside the host organism. Since specific and living host cells are not present in the environment, the number of viral particles cannot increase and the amount of any contaminating viruses should decline over the storage time. However, foodborne and waterborne viruses are infectious in very low doses (Carter, 2005; Vasickova *et al.*, 2005).

Virus survival outside the host organism (i.e., the integrity of the viral genome, capsid and envelope) is affected by a combination of various environmental conditions and biological, physical and chemical factors (Carter, 2005; Fig. 9.1).

The persistence of viral particles can be predicted primarily according to the virus classification. However, variations in virus survival occur within a given virus family or even genus. Based on its similar classification and thus similar genomic organisation and physicochemical characterisation, feline calicivirus (FCV) is used as a surrogate virus for non-cultivable NoV. It has been found, however, that human NoVs are more resistant than FCV to low and high pH and to other environmental factors (Duizer *et al.*, 2004; Hewitt and Greening, 2004). The coronaviruses OC43 and 229E represent an example of variation within the same genus. The infectivity of these two viruses differs temporally: coronavirus 229E was detectable after 3 h on various surfaces, while coronavirus OC43 persisted for only 1 h or less (Sizun *et al.*, 2000).
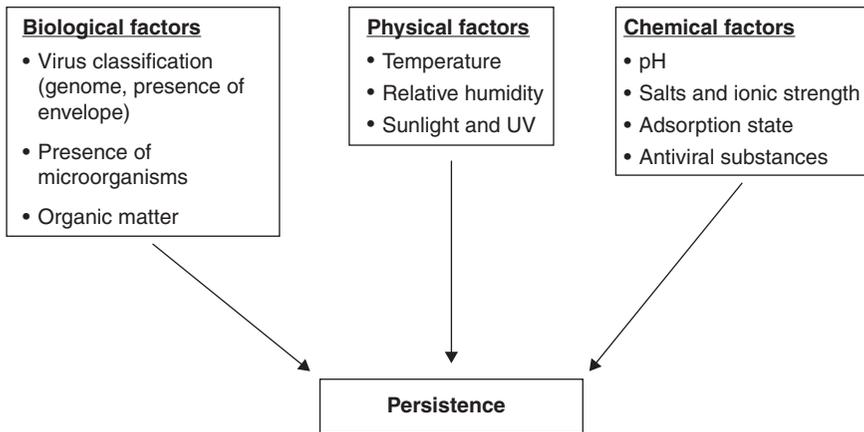
Fig. 9.1 General factors affecting virus persistence in the environment.

It is generally known that non-enveloped viruses have a higher resistance to desiccation and thus are spread more easily than viruses with an envelope, which corresponds also with their mode of transmission. Enteric viruses produced in either the intestines or livers of infected humans or animals are relatively stable outside the host organism. They are able to withstand a pH as low as 3 for a certain period, which allows them to pass through the stomach and cause infection in the small intestine or liver. In contrast, enveloped non-enteric viruses such as respiratory viruses are less often transmitted via contaminated food or surfaces and are generally less stable in the environment (Duizer *et al.*, 2004; Howie *et al.*, 2008).

The biological factors affecting viral persistence also include other micro-organisms present in the environment. Bacteria or microscopic fungi can attack and inactivate viral particles by direct or indirect actions. For example, micro-organisms produce metabolites such as proteolytic enzymes, which adversely affect viral capsid. In fact, some environmental isolates of bacteria can use viral proteins and nucleic acids as a nutrient source. Also, certain bacteria can produce substances with antiviral activity, but whose molecular weight is so low that they cannot act enzymatically (Cliver and Hermann, 1972; Deng and Cliver, 1992; Deng and Cliver, 1995). In contrast, an increasing number of microbes have been described that can protect viruses from desiccation or disinfection (Storey and Ashbolt, 2001, 2003). Various studies have suggested that infectious viral particles may be trapped and thus accumulate in biofilms. Although the rate of contamination of biofilms with pathogenic viruses could be low and even though this attachment was observed only experimentally, biofilms should be considered as protective reservoirs of pathogenic viruses and could be implicated in numerous persistent viral infections (Lacroix-Gueu *et al.*, 2005).

Notwithstanding all of the above, the degree of virus persistence is mostly affected by temperature, with relative humidity (RH) also being an influencing factor. However, infectious viral particles are able to persist for days or even months over a range of temperatures and RH. Although viruses are destroyed by boiling, the thermal stability of some is remarkable (Carter, 2005). Temperatures of above 90°C are required for a 2 $\log_{10}$ inactivation of HAV in shellfish extract (Millard *et al.*, 1987), and a heat treatment of 100°C for 2 min has been recommended (Croci *et al.*, 1999) for elimination of infectious HAV in shellfish. On the other hand, viruses are preserved by refrigeration or freezing, which could cause problems, particularly with regard to food. The effect of RH is variable within virus types. It is believed that viruses with higher lipid contents (enveloped viruses) tend to be more resistant to lower RH and non-enveloped viruses are more stable at higher RH; that is, the survival of enveloped viruses is better when the RH is lower than 50%, while an RH level of higher than 80% is more beneficial for the persistence of non-enveloped viruses. However, several exceptions exist. In general, reducing the temperature promotes virus survival, but viruses might respond differently to RH (Mbithi *et al.*, 1991).

Sunlight and ultraviolet radiation (UV) are also important factors promoting the inactivation of viruses (Sagripanti and Lytle, 2007). UV light primarily not only targets viral nucleic acids but can also modify proteins of the viral capsid. Virus resistance to UV is influenced by virus type. Viruses with double-stranded genomes (dsRNA or dsDNA) are more resistant against UV inactivation than viruses with genomes consisting of single-stranded nucleic acid (ssRNA or ssDNA). Viral resistance to UV also depends on the size of the genome; it was found that the rate of nucleic acid degradation increased linearly with increasing fragment size of nucleic acid (Gerba *et al.*, 2002; Hijnen *et al.*, 2006; Tseng and Li, 2007). According to Hijnen *et al.* (2006), adenoviruses are the most resistant to UV. The sensitivities of FCV, rotaviruses, poliovirus and coxsackievirus were found to be similar, and HAV was the most sensitive virus (Hijnen *et al.*, 2006). The effect of UV-B was less pronounced on surrogate caliciviruses (enteric canine calicivirus and respiratory FCV) than on bacteria (De Roda Husman *et al.*, 2004) and enteroviruses (Gerba *et al.*, 2002) and it was more effective against phage MS2 (De Roda Husman *et al.*, 2004), adenoviruses (Gerba *et al.*, 2002) and *Bacillus subtilis* spores (Chang *et al.*, 1985).

Due to the mode of infection, foodborne and waterborne viruses are able to survive the extremes of the gastrointestinal tract. In general, viruses are most stable at pH values close to 7 and prefer low pHs (3–5) rather than alkaline pHs; 9–12 (Vasickova *et al.*, 2010). The pH can also indirectly affect virus persistence in the environment when persistence is influenced by the virus adsorption on solid particles or surfaces (Gerba, 1984). Persistence is increased while the viral particles are immobilised (adsorbed) and viruses are able to keep their infectivity after desorption. The interactions that take place between the viral particles and the adsorbent surface are determined by

their characteristics and involve electrostatic and hydrophobic interactions and ionic strength. Due to the fact that the surface charge of viral particles is related to the pH of the environment, any disruption of such interactions is connected with pH changes (Gerba, 1984). Viral adsorption is theoretically better at high ionic strengths. Therefore, salt solutions are commonly used to promote adsorption. Viruses can also be stabilised and protected by dissolved, colloidal and solid organic matter including faecal and humic material. Organic matter has a low isoelectric point and thus carries a negative surface charge at most pH levels (Boone and Gerba, 2007). Besides, some viruses have more than one isoelectric point (Michen and Graule, 2010).

Comprehensive information regarding the influence of the environment on all viruses and their stability under external conditions does not exist. Most studies have used only a few target viruses or their surrogates and have not considered the effects of a combination of treatments or factors. Since experimental conditions or methods vary and studies performed to date have yielded contradictory results, it is difficult to draw conclusions from these experiments (Carter, 2005). Because the majority of foodborne or waterborne viruses which infect humans cannot be cultivated routinely, survival data and inactivation rates are sparse.

9.4   Persistence in aquatic environments

Foodborne and waterborne viruses can be present in any kind of untreated water, due to contamination caused by faecal material of human or animal origin. Despite the possible dilution of faecal contamination, the evidence suggests that viruses can persist in water in sufficient amounts to cause disease (Seymour and Appleton, 2001). In addition, it was found that these viruses might survive wastewater treatment and thus pose a threat to recreational users, consumers of shellfish or consumers of fresh fruit and vegetables (Carter, 2005). Viruses present in untreated water are inactivated slowly by a combination of the biological, physical and chemical effects mentioned above. It appears that the temperature of contaminated water and virus type are the most important factors affecting persistence (Seymour and Appleton, 2001). It was found that outbreaks caused by NoV are much more prevalent in the winter than in the summer, which is possibly due to lower temperatures (Doultree et al., 1999; Mattison et al., 2007) promoting survival.

Virus persistence in water can vary widely; the time required for a reduction of 1 log titre of enteroviruses ranges between 14 and 288 h (Chung and Sobsey, 1993; Callahan et al., 1995). These variations could reflect the different conditions under which the experiments and studies were performed. Generally, viruses are capable of persisting for weeks or months at environmental temperatures and when sheltered from UV in combination with low temperature can even survive for years (Carter, 2005). HAV and poliovirus were shown to persist for more than 1 year in mineral water stored at 4°C

while they remained infectious for 90 days at 10°C in wastewater and ground water (Biziagos *et al.*, 1988). According to Sobsey *et al.* (1988), HAV can survive in fresh or salt water for up to a year. No significant loss of infectious rotavirus particles was observed after 64 days at 4°C in raw water, treated tap water or filtered water, but a 99% reduction of titre was observed after 10 days at 20°C (Raphael *et al.*, 1985). Further studies have demonstrated the persistence of rotaviruses and poliovirus for more than 1 year (Biziagos *et al*, 1988) and of adenoviruses 40 and 41 for 300 days in artificially contaminated water (Enriquez *et al.*, 1995). Infectious astroviruses have been detected in drinking water after 90 days at 4°C (Abad *et al.*, 1997).

The rate of virus inactivation at or below 30°C is dependent on the pH and the ionic composition of the water environment (Salo and Cliver, 1976). It was previously thought that inactivation is associated mostly with genome degradation (Dimmock, 1967 as quoted by Cliver, 2009b), but subsequent studies demonstrated that viruses can be inactivated due to the loss of the ability to attach to host cell receptors, which implies a subtle denaturation of capsid proteins (Nuanualsuwan and Cliver, 2003). The pH and presence of salts do not appear to have a significant direct effect on virus persistence in natural waters, but instead influence the interaction between viruses and solid particles present in water (Seymour and Appleton, 2001). Several studies have suggested that adsorption of viral particles to particulate matter and sediments can result in substantial protection against inactivation procedures in the water environment (Mandel, 1971 as quoted by Cliver, 2009b). It was found that enteric viruses are destabilised and subsequently inactivated in water which is poor in salt ions, for example, Mg^{2+}. In contrast, increased concentrations of salts (NaCl) could be virucidal for several kinds of viruses (Vasickova *et al.*, 2010).

Viruses have been shown to persist better in sewage-polluted water than in non-polluted water environments, probably due to the presence of organic matter or solid particles. Alvarez *et al.* (2000) reported that the inactivation of bacteriophage MS2 and poliovirus in pre-filtered ground water was faster than in raw ground water. On the other hand, micro-organisms normally present in fresh and sea water can play an important role in the inactivation of viruses. The study by Gordon and Toze (2003) showed reduced inactivation rates in ground water in the absence of bacteria. Bosch (1995) demonstrated antiviral activity of bacteria present in sea water. However, bacteria can also have protective effects on viruses. Recent studies have revealed that viral particles are able to penetrate biofilms and in this way benefit from protection against environmental stress such as desiccation or other effects of antimicrobial agents. In addition, during biofilm erosion or sloughing, protected immobilised viral particles may be released into the environment, subsequently contacting their target host organism (Lehtola *et al.*, 2007; Briandet *et al.*, 2008; Helmi *et al.*, 2008). Although the protective effects of biofilms were observed only experimentally and natural biofilms can be contaminated with only a very low dose of viruses, biofilms should be considered as a protective

reservoir for pathogenic viruses and facilitate numerous persistent viral infections (Lacroix-Gueu *et al.*, 2005).

As mentioned above, enteric viruses may survive the treatment of wastewater. Most enteric viruses (e.g., HAV, NoV, adenoviruses and HEV), have been found in wastewater and subsequently in treated water using PCR, and the detection of viruses in sewage systems has been conducted using the cultivation of some viruses on cell lines (Gantzer *et al.*, 1998; Matsuura *et al.*, 2000; Sedmak *et al.*, 2005). Astroviruses have been found in sewage treatment plant inlet and effluent waters. A reduction of approximately 2 log of viruses was detected in response to waste water processing and 10^5 copies of the astrovirus genome (per 1 l) were found in effluent water (Le Cann *et al.*, 2004). In addition, intact enteroviruses and HAV were detected in sludge originating from a wastewater plant (Albert and Schwarzbrod, 1991; Graff *et al.*, 1993).

Enteric viruses have also been detected in drinking water (Payment, 1989), which may in large part be due to contamination of water sources, failures in the treatment process (i.e., pressure failure, insufficient disinfection or exceptionally high concentration of pathogenic viruses), or contamination of an already treated water source (Carter, 2005). Filtration together with subsequent disinfection (e.g., chlorination, ozone or UV treatment) of raw water can achieve up to a 10 000-fold reduction in contaminating agents. During the disinfection procedure, turbidity has the greatest effect on virus survival. It was found that increasing the turbidity could decrease the effect of free chlorine, shield the viruses from UV and promote virus aggregation. It is assumed that the procedures used commonly for drinking water treatment might not destroy all viruses (Le Chevallier *et al.*, 1981). The study by Gofti-Laroche *et al.* (2003) showed a correlation between the presence of astrovirus RNA in drinking water and increased risk of intestinal disease. The results of a volunteer study suggested that NoVs could survive some water chlorination (Keswick *et al.*, 1985). In contrast, rotavirus is inactivated efficiently by chlorine (Carter, 2005).

Water plays an important role not only in the spread of human pathogenic viruses. Waterborne outbreaks of enteric viruses are common and thus interest has recently been focused on virus survival in drinking water and wastewater. The contamination of water sources is also a crucial step determining the potential of viruses to contaminate soil or crops. Although a number of experiments have been performed to investigate this issue, there is still a lack of information regarding the persistence of these viruses in the water environment and thus about their ecology. Furthermore, variations in the results of studies of virus survival in water emphasise the need for experiments to be performed under comparable conditions using standardised methods.

9.5 Persistence in soils

Predictably, viruses and bacteria are more abundant in a diverse range of moist soil types compared to dry and arid soil (Srinivasiah *et al.*, 2008).

Foodborne or waterborne viruses may contaminate soil via land disposal of sewage sludge or already contaminated water. Prolonged persistence has also been shown in such environments. Hurst *et al.* (1980) investigated the effects of several environmental conditions on virus persistence in soil. Based on the results, temperature, soil moisture content, degree of virus adsorption to soil, soil levels of resin-extractable phosphorus, exchangeable aluminium and the pH of the soil were found to influence virus persistence. Temperature and virus adsorption appear to be the most important factors affecting virus persistence in soil (Hurst *et al.*, 1980).

Infectious poliovirus was detected in spray-irrigated soil after 96 days during the winter season, while a maximum survival period of 11 days was demonstrated during the summer time (Tierney *et al.*, 1977). Oron *et al.* (1995) found that a relatively high soil temperature (30°C) together with low moisture content hindered poliovirus survival. Infectious particles of poliovirus and echovirus were recovered from loamy soil (pH 7.5) after 110–130 days at 3–10°C, while at 18–23°C the duration of persistence fell to 40–90 days (Bagdasaryan, 1964 as quoted by Rzezutka and Cook, 2004). Damgaard-Larsen *et al.* (1977) studied the persistence of enteroviruses in sludge-amended soil, where the temperature of the environment varied between −12°C and 26°C. A loss of 0.5–1 $\log^{10}$ of viral titre was observed per month, and viruses were still detected after 6 months of monitoring. In general, viruses persist for longer periods of time at low temperatures (4–8°C) than higher temperatures (20–37°C).

Wet conditions are usually associated with low soil temperatures. Poliovirus persistence was found to increase as more liquid was added to soil beyond the saturation point and then decrease as the soil moisture content increased up to the soil saturation point; that is, the inactivation rate for poliovirus increased when the water content of sandy soil increased from 5% to 15% and subsequently decreased when the water content further increased to 25% (Hurst *et al.*, 1980). It was also shown that virus survival is apparently prolonged in anaerobic conditions. The effect of soil water content on virus inactivation is dependent on soil type (Zhao *et al.*, 2008). Yeager and O'Brien (1979) found that viruses are able to persist for at least 180 days in saturated sandy loam or sandy soil, while no infectious viral particles were detected in dried soil regardless of soil type after 25 days.

Phosphorus, aluminium and pH have indirect effects on virus survival via their influence on the adsorption state. Whilst the presence of aluminium increases the virus adsorption rate, the level of resin-extractable phosphorus (phosphate anions) results in the elution of the adsorbed viral particles from soil. Virus adsorption on soil particles is increased when the pH of soil decreases and higher pH values result in the release of virus from soil particles (Zhao *et al.*, 2008). Virus survival is likely to be highest in types of soil that would be most effective in preventing ground water contamination. The study of Sobsey *et al.* (1980) compared the interactions of different soil materials and two different virus types (poliovirus type 1 and reovirus type 3). The

behaviour of both viruses was found to be similar, which is in contrast to the studies of Goyal and Gerba (1979) and Landry *et al.* (1979), who found that adsorption varies with virus type and even strain. Generally, clay materials efficiently adsorbed viruses from waste water over a range of pH values, while sands and organic soil materials were poorer adsorbents; their ability to adsorb viruses increased only at low pHs together with the addition of total dissolved solids or divalent ions (Sobsey *et al.*, 1980). A further study confirmed that the presence of clay mineral enhances the persistence of viruses (Vettori *et al.*, 2000). Sobsey *et al.* (1980) also reported that even under unsaturated conditions viruses could still be washed from sandy soil and were able to contaminate water sources during heavy rainfall.

Virus survival in soil also appears to be generally greater under sterile than non-sterile conditions, suggesting the influence of other micro-organisms on virus survival in soil (Nasser *et al.*, 2002). The presence of aerobic bacteria could decrease virus survival due to the production of proteolytic substances. However, a study by Hurst *et al.* (1980) did not confirm this hypothesis: virus survival was not significantly affected by the addition of sewage effluent.

Due to the increasing land application of wastewater, it is important to evaluate the influence of different factors on virus survival in soil and thus evaluate the risk of resultant human illness. In addition, virus adsorption and inactivation in soil are crucial steps determining the potential of viruses to contaminate water resources (Zhao *et al.*, 2008). Viruses from sewage do not bind readily with soil particles and thus they can easily enter ground waters and in this way contaminate water sources (Seymour and Appleton, 2001). Furthermore, studies with poliovirus suggested that viral particles can infiltrate the roots and body of tomato plants (leaves) from the soil (Oron *et al.*, 1995). However, there is no evidence of illness from this source. To date, only a few studies have examined the biological factors affecting virus persistence in soil. Therefore, further studies are needed to evaluate the complex interactions between viral particles, soil and autochthonous micro-organisms; due to the restrictions of laboratory conditions the study of viruses under natural conditions, that is, in the field, should be emphasised.

9.6 Persistence on food-related surfaces

Surfaces can be contaminated directly through contact with body secretions and fluids containing infectious viral particles or indirectly via the aerosol or other contaminated fomites. Once a surface is contaminated, it may serve as a source of infectious viral particles for animate and inanimate subjects; for example, contaminated door handles or hands were found to be an efficient vector of viruses. It has been reported that at least 14 persons could be infected or their hands contaminated by touching a polluted door handle. Successive transmission of infectious viral particles from one person to

another could be followed for up to six contacts via contaminated hands (von Rheinbaben *et al.*, 2000). The main factors influencing such kinds of transmission are temperature, RH, adsorption state and the character of the surface (Vasickova *et al.*, 2010).

Studies based on surrogate viruses indicate that NoV can persist for prolonged periods at low temperatures and that it can then be transmitted by different environmental matrices such as surfaces (Mattison *et al.*, 2007). It was found that FCV dried onto a glass surface and stored at 4°C displayed a 4.75 log reduction over 56 days, while the virus titre declined to undetectable levels at room temperature over 21–28 days and the infectious virions were not detectable at 37°C after 1 day (Doultree *et al.*, 1999). Studies focused on other viruses showed similar results. The infectivity of rotaviruses decreases more rapidly at 37°C than at 20°C or 4°C. Approximately 10% of rotavirus particles remain infectious at 4°C (RH 25–50%) after 10 days, while less than 1% of infectious virions persist at 20°C after 2 days (Moe and Shirley, 1982). A study by Abad *et al.* (1994) found that enteric viruses, including HAV and rotavirus, can persist for extended periods (up to 30 days) on fomites, and virus survival was prolonged at 4°C compared to 20°C. Astroviruses also showed a faster rate of inactivation at 20°C compared to 4°C (Abad *et al.*, 2001). Overall, astroviruses can persist longer than poliovirus (as a representative of enteroviruses) and adenoviruses; however, they show less robust survival than HAV or rotavirus (Mbithi *et al.*, 1991).

When the effect of RH on the survival of viruses was studied, it was found that RH had little effect on virus persistence at 5°C, while at 20°C viruses were able to survive for longer periods in low RH (Sattar *et al.*, 1987; Bidawid *et al.*, 2000). Mbithi *et al.* (1991) demonstrated that 34% and 52% of HAV particles remained infectious at high RH (80%) and low RH (25%) after 4 h at 20°C on a non-porous surface, respectively. In contrast, Abad *et al.* (1994) reported that HAV persistence was enhanced at high RH (90%) in comparison with moderate RH (50%) on a non-porous material after 60 days of storage at 20°C. Enhanced survival of rotavirus was observed at high RH on porous surfaces (Abad *et al.*, 1994), while Sattar *et al.* (1986) reported better rotavirus survival in low and medium RH on non-porous ones. Based on these results, virus persistence on surfaces is mainly related to virus strain, type of surface and temperature. The results with regard to the effect of RH are contradictory.

Foodborne viruses are potentially resistant to drying: 7% of rotavirus and 16–30% of HAV infectious particles persisted on finger pads after drying at room temperature for 4 h, although 68% of virions lost their infectivity within the first hour of the experiment (Ansari *et al.*, 1988; Mbithi *et al.*, 1992). It was also demonstrated that NoVs could be transferred from a contaminated surface to clean hands and via contaminated hands could cross-contaminate a series of seven surfaces (Barker *et al.*, 2004). The infectivity of NoVs is complicated by their problematic cultivation. A protective effect of organic

material was demonstrated by Lee *et al*. (2008): a 2.7 $\log^{10}$ reduction of murine norovirus (MNV; surrogate of NoV) was detected at 18°C in a stool suspension, compared to 5.3 $\log^{10}$ on gauze or diaper surface. On non-porous surfaces, poliovirus and adenovirus persisted better in the presence of stool than did HAV and rotavirus. In contrast, the presence of stool material had a negative influence on the survival of poliovirus and adenovirus on porous fomites. The presence of food residues on steel material increased the persistence of MNV; a decline of 6.2 log and 1.4 log was observed at day 30 in residue-free and residua-present fomites, respectively (Takahashi *et al*., 2011). The bacteria present in organic matter produce certain virucidal substances and thus are able to inactivate viruses (see section 9.3). This kind of inactivation is also dependent on temperature: the lower the temperature, the lower the activity of other micro-organisms and the longer the virus is able to maintain infectivity (Deng and Cliver, 1995). On the other hand, protective effects of bacterial reactions on viruses and bacterial biofilms have been reported and micro-organisms could have protective effects on virus survival due to their production of biofilms (see section 9.4).

The relationship between virus persistence and adsorption state is influenced by characteristics of both the virus and the type of surface. The majority of viruses remain viable for a longer period on non-porous materials (Tiwari *et al*., 2006; Boone and Gerba, 2007; Lamhoujeb *et al*., 2008); however, there are also several exceptions. Astroviruses are able to remain infectious at 4°C (RH 90 ± 5%) for 60 days when adsorbed on non-porous and for 90 days on porous surfaces; poliovirus and adenovirus persist also longer on porous (paper and cotton towel) than on non-porous material (aluminium, china, glazed tile, latex and polystyrene). Obviously, the physical properties of the surface may inhibit the recovery of viral particles from surfaces. Viruses can be trapped within the matrix, especially within the porous surface, and thus the results of virus persistence could be misinterpreted. Furthermore, several studies indicate virucidal activity of surfaces such as aluminium or copper. Adenovirus, poliovirus and the B40-8 phage showed lower persistence on aluminium than on other non-porous material (Thurman and Gerba, 1988; Abad *et al*., 1997). The antimicrobial properties of copper and copper-based surfaces were also demonstrated (Faundez *et al*., 2004; Noyce *et al*., 2007).

The transmission of pathogenic viruses via contaminated surfaces is clear, but comparable data concerning the persistence of viral particles on surfaces is still lacking and studies performed to date have yielded contradictory results. Virological monitoring as well as studies of virus persistence on surfaces, using comparable methods and natural conditions, would be very useful in assessing the risks of virus spread via contaminated surfaces. In addition, information about the presence of non-cultivable viruses (NoV or HEV) on surfaces and hence their transmission via contaminated surfaces are still based on surrogate viruses (von Rheinbaben *et al*., 2000; Boone and Gerba, 2007).

9.7 Persistence in food

If food cannot be reliably decontaminated during production, adequate food preparation such as cooking becomes critical (Cliver, 2009b). The consumption of food which is processed only minimally before consumption or served raw such as fruit and vegetables, shellfish or some traditional meat specialities represents a considerable risk (Vasickova *et al.*, 2005). Many studies have shown that the washing of already contaminated fruit and vegetables is not sufficient and that the depuration of virus-contaminated shellfish is highly unreliable (Croci *et al.*, 1999; Dawson *et al.*, 2005). Most of these studies were focused on predicting the persistence of foodborne viruses or their surrogates in cases of non-cultivability. Despite the variability in experimental conditions, several conclusions can be drawn. Virus persistence in fresh food is primarily influenced by the surrounding temperature, RH and the characteristics of the food and surrounding environment. Studies indicate that viruses are able to persist well on chilled, acidified, frozen foods and foods packed under modified atmosphere or in dry conditions even when preservation methods such as high hydrostatic pressure processing and irradiation were used. The virus viability was found to usually exceed the shelf-life of fresh food. Further, the decontamination of fresh produce can cause a reduction of at most 1 to 3 log in infectious viral particles even when chlorine and peroxyacetic acid solutions are used (Gulati *et al.*, 2001; Baert *et al.*, 2009).

Although viruses can be rapidly diluted in water, virus concentration can greatly increase in shellfish due to their filter-feeding; levels can be 100–1000-fold higher than in the surrounding water (Carter, 2005). According to DiGirolamo *et al.* (1970) and Tierney *et al.* (1982), viruses can persist well in shellfish; no loss of viral infectivity was observed over a month's refrigerated storage or 4 months when frozen. Ueki *et al.* (2007) compared the persistence of MNV and NoV in the digestive tissues of oysters and after depuration for 10 days. FCV was completely depleted, while NoV still persisted. Hewitt and Greening (2004) inoculated commercially prepared marinated mussels with HAV and FCV. A 1.7 log reduction in HAV was observed after 4 weeks of storage at 4°C and a 7 log decrease in MNV was seen after 1 week of storage under the same conditions.

The persistence of enteric viruses has been determined in a range of different fruits and vegetables (Seymour and Appleton, 2001). Konowalchuk *et al.* (1974) found no significant loss in coxsackievirus titre in lettuce stored for 16 days under moist conditions at 4°C, but inactivation was observed during storage in dry conditions. Badawy *et al.* (1985) reported that a cultivable strain of rotavirus (SA-11) is able to survive on lettuce, carrot and radishes for up to 30 days at 4°C. However, virus inactivation was greater at room temperature, and it was found that the virus still remained infectious after 25 days. These results contradict the documented persistence of HAV (Stine *et al.*, 2005). It was found that HAV survived longer on lettuce in medium RH (45.1–48.4%), compared to high RH (85.7–90.3%) (Abad *et al.*, 1994). In general, chilled

storage (2–8°C) retards respiration, senescence, product browning, moisture loss and microbial growth in fruit and vegetables, but contributes to virus persistence (Seymour and Appleton, 2001). Besides, based on the inactivation rates calculated in the study by Stine *et al*. (2005), a 99.9% reduction in HAV-infectious particles could require as much as 822 days in pre-harvest lettuce.

According to Vega *et al*. (2008), it seems that electrostatic forces play the major role in virus adsorption to lettuce. Mattison *et al*. (2007) suggested that smooth surfaces such as those of lettuce might provide less protection to the viral particles compared to ham; ham is rich in proteins and fats, which might protect virions against dryness and other factors.

Several studies indicate that on plant surfaces viruses can be exposed to potentially virucidal substances, such as organic acids, phenols, ethanol or acetaldehyde, which could accelerate the inactivation of virions (Lamhoujeb *et al*., 2008). The presence of such substances was described in several kinds of fruits and vegetables. Significant differences in viral recovery were found for strawberries, cherries and peaches kept in a humid atmosphere at 4°C; virus survival was lower than on other studied fruit (Konowalchuk and Speirs, 1975). Kurdziel *et al*. (2001) reported 1 log reduction of poliovirus after 11.6 days of storage for lettuce and after 14.2 days for white cabbage, while no significant decline in virus titre was observed on green onions after 15 days. A longer survival of HAV was detected on lettuce than on fennel and carrots (Baert *et al.*, 2009). A faster decline of HAV was also observed on fennel and carrots, which was reported to be due to the antimicrobial activity of carrot extracts (Babic *et al*., 1994). Similar effects regarding poliovirus inactivation were also found for grape juice, apple juice and tea (Konowalchuk and Speirs, 1976). These findings could also be connected with the low pH of such food products. Despite this, outbreaks of hepatitis A have been associated with several kinds of fruit and fruit juices (Seymour and Appleton, 2001).

Freezing has a minimal effect on enteric viruses in berries (strawberries, blueberries and raspberries) and herbs (parsley and basil). The infectivity of NoV, HAV and rotavirus was not reduced significantly after freezing for 3 months; the number of infectious FCV decreased in frozen raspberries and strawberries, however (Butot *et al*., 2008). A reduction (<2 $\log^{10}$) in infectious poliovirus on frozen strawberries was also reported after 15 days of storage (Kurdziel *et al*., 2001), which could be explained by the low pH values of this fruit or the presence of virucidal substances. Deep-frozen storage of onions and spinach for 6 months had no effect on MNV survival (Baert *et al*., 2008). On the other hand, FCV and canine calicivirus showed declines in infectivity of 0.34 log and 0.44 log after 5 cycles of freeze-thawing (Duizer *et al*., 2004).

Despite the number of studies regarding the persistence of viruses in food, much remains to be learned (Cliver, 2009b), especially because of the absence of methods for the detection of all types of infectious viral particles. To resolve this problem predictive models have been proposed for persistence in shellfish which were based on surrogate viruses such as MNV and

a cell culture-adapted HAV strain (Buckow *et al.*, 2008; Grove *et al.*, 2009). However, owing to the problems with surrogates, such models are not likely to portray the inactivation of pathogenic viruses in food accurately and the data obtained should be presented only as preliminary evidence of how pathogens might respond to different conditions (Richards, 2012).

9.8 Acknowledgement

9.9 References

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