Overview of viral persistence on food surfaces

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ABSTRACT
Interest in viruses in food products stems from the large number of outbreaks of acute gastroenteritis among persons of all ages. The frequency and extent of these outbreaks may still be overlooked, mainly because of the lack of means of easy diagnosis. Basic information about viral epidemiology, factors determining the attachment of viruses to food surfaces, viral inactivation under certain conditions and persistence on different food products is still being acquired. An important aspect of the transmission of viruses is the length of time for which these pathogens can stay on different surfaces or matrices. Understanding of the conditions that promote the persistence of viruses as infectious agents in the environment would help to eliminate viruses from the food supply chain. Factors such as ambient pH, temperature and humidity, surface topology (food or inanimate surfaces) may play major role in virus persistence and resistance to chemical treatment and preservation methods as well as the attachment to various receptors or surface components. Investigations have focused on these factors using laboratory-adapted strains or surrogates that can be propagated in vitro. The aim of this review is to compile the recent findings regarding the factors that promote the persistence of foodborne viruses on environmental and food surfaces.

KEYWORDS: viral persistence, food surfaces, HAV, norovirus

INTRODUCTION
It is well known that viruses are able to persist for long time as infectious agents in environments far removed from the living host cells necessary for their replication. The survival of viruses in the environment is therefore greatly supported by interactions with commodities that allow them to remain in the environment or to be transmitted to a host for replication. Some of the factors that influence the environmental stability of viruses include relative humidity, temperature and the type of surface onto which they have been deposited. The likelihood that a virus will be transmitted from the environment to a host depends on the topography, material type and coefficient of friction of the surface, the time and pressure involved in the contact between the virus and the surface, the amount of virus present and the nature of its attachment to the surface. In the food chain, the most common commodities that have been linked to viral survival are inanimate surfaces used in a food service setting or food products. In the food-processing environment, viral transmission from non-porous surfaces such as stainless steel, plastic and glass has been correlated with poor sanitation techniques and the ability of the virus to interact with the surface. Among the wide variety of food commodities in the market, certain products are more suitable to endure the presence of viruses for longer periods of time. Therefore, the interactions that a virion establishes with a food component on the surface

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are crucial for its persistence. The elucidation of these interactions, which may be specific for each type of foodstuff, would provide a basis for the elimination of viruses from the food supply.

Some published studies have focused on the resistance of viruses in the environment which are commonly associated with fomites and soil [79] or with water [41]. Diverse factors have promoted new viruses or re-emerging viruses to be more commonly found in certain food products. The development of molecular techniques has made possible the detection of viruses that could not be detected by other means or propagated in vitro. The application of new processing technologies has created opportunities for the emergence or re-emergence of other viruses in foods. Finally, the increasing popularity of raw or ready-to-eat foods that may be produced not only locally but globally is creating additional opportunities for viruses as well. The globalization of the food chain is one of the main challenges for food safety due to the possibility of introducing pathogens such viruses to food distribution channels in which they were previously rare or absent. Consequently, the persistence of viruses commonly found in foods has recently gained more attention in an effort to understand the interactions between viruses and foodstuffs and ultimately to improve the safety of the food chain. Current understanding of the persistence of viruses in the environment and in foods remains limited. In this review, we compile and examine the findings of recent studies relating to the most common viruses or their surrogates in food matrices.

Most significant foodborne viruses

The diversity of factors contributing to the recent emergence and re-emergence of foodborne pathogens has prompted a call to explore several approaches to improving food safety. These include the development and implementation of HACCP programs, the development of strategies for eliminating microorganisms from food and the enhancement of current pathogen detection methods [100]. The latter is important for assembling epidemiological data, which can be used to increase knowledge and stimulate interest in food safety, establish risk reduction strategies and evaluate the effectiveness of food safety programs. Advances in areas such as virology and molecular biology have facilitated the detection of viruses and consequently uncovered some of their transmission modes. In addition, the development of surveillance systems has helped to understand more about the viruses most frequently associated with foods. One of the major surveillance systems is the Global Foodborne Infections Network, which has made great strides in estimating the global foodborne burden. This collaborative monitoring initiative of the World Health Organization has brought together partners such as the Public Health Agency of Canada, the US Centers for Disease Control and Prevention, the European Centre for Disease Prevention, the Danish National Food Institute, Institut Pasteur, Utrecht University, OzFoodNet, PulseNet International and the Japan National Institute of Public Health. This program has made possible to estimate that 2 million people die of diarrhoea every year [99]. From these numbers, it has been inferred that viruses play a major role in outbreaks of illness involving ready-to-eat foods or foods that are eaten raw. Recent data point to prepared salads as one of the main vehicles of norovirus outbreaks in England and Wales from 1996-2007, frozen raspberries in similar outbreaks in Denmark, Sweden and France and lettuce as vehicle of hepatitis A virus (HAV) transmission in Denmark [57]. Furthermore, the key role of food workers in the spread of illness has been assessed in HAV and norovirus outbreaks in which over 50% of the cases were hospitalized. Certain outbreaks of HAV have caused numerous deaths, making this virus a bigger health risk than other pathogens such as Salmonella typhi, Shigella sonnei or Staphylococcus aureus [89]. Even though other viruses such as human rotavirus, astrovirus, sapovirus, aichi virus, coronaviruses, enterovirus and adenovirus appear capable of causing foodborne illness or emerging as foodborne pathogens, all recent data show that norovirus and HAV continue to be the most common viral agents of foodborne illness [102]. Most studies of viral persistence on agrifood surfaces therefore have been carried out with these two viruses.
**Hepatitis A virus**

HAV has long been recognized as a significant causative agent of disease associated with food but knowledge about its behavior and stability in the environment has been obtained in relatively recent years [53, 80]. Since the early 1900s, it was determined that HAV could be transmitted by food, person-to-person contact and water. HAV is associated mainly with consumption of shellfish and fresh produce [24]. HAV contamination of a food product can occur at any point during cultivation, harvesting, processing, distribution, or preparation. Some aspects of foodborne transmission have been recognized but remain difficult to establish due to i) the inability of patients to recall food consumption patterns during the 2–6 weeks before the onset of illness, ii) contaminated food may be present only in some areas, iii) problems in collecting naturally contaminated food samples and iv) cases may increase gradually or not be reported [24].

Unlike other *Picornaviridae* strains, HAV is stable at pH 1, resistant to heat (56°C for 30 min) and does not show cross hybridization with enteroviruses, rhinoviruses or other picornaviruses [20]. The virion has a density of 1.33 g/cm³ in CsCl and a sedimentation coefficient of 156 as determined by ultracentrifugation [67]. The non-enveloped icosahedral virion was first adapted to cell culture in 1979 and its diameter was later determined to be 27 to 32 nm [20]. HAV belongs to the genus *Hepatovirus* of the *Picornaviridae* family. The family *Picornaviridae* contains a group of human and animal pathogens that are closely related in virion structure and genome and share similar mechanisms of propagation. However, HAV replicates slowly and requires a long adaptation period to grow in cell culture, thus differing from other members of the family [70]. Most human HAV strains belong to type I or III such as the strains adapted to laboratory conditions, namely HM-175 originally isolated in Melbourne, Australia and CR326 from Costa Rica, both of which are type-I strains. The organization of the single-stranded RNA genome within a capsid of icosahedral symmetry is similar to that of other picornaviruses. The positive-sense RNA is 7.5 kb in length and consists of a 5’ untranslated region, a coding region of 2,225 to 2,227 nucleotides and a 3’ untranslated region (Figure 1). The HAV genome is highly conserved but enough genetic diversity exists to define several genotypes and subgenotypes. The molecular and biological properties exhibited by HAV distinguish this virus from other picornaviruses. In molecular terms, HAV presents three differences from other picornaviruses, i) the structure of the HAV internal ribosome entry sequence, which shows a low efficiency in directing translation, ii) the 3C protease, which is the only protease involved in the primary and secondary cleavage of the viral polyprotein and iii) the codon usage, which has been suggested as a strategy to avoid competition for tRNAs in the absence of a specific shutoff mechanism of cellular protein synthesis [75]. These three properties of the HAV virion appear to contribute to highly accurate folding of the capsid and low capsid variability and consequently to a particle that is very stable in the environment and resistant to low pH and detergents [75].

Improved living conditions and sanitation as well as the availability of vaccine have contributed to a significant decrease in the frequency of HAV cases among populations of high socio-economic status in developing countries and in most of the

![Figure 1. Organization of the picornavirus genome. The single ORF is divided into three regions: P1 encodes four structural proteins (VP1–VP4), while P2 (2A–2C) and P3 (3A–3D) encode seven non-structural proteins (adapted from Kok and McMinn, 2009).](image-url)
population in developed countries [73]. Nevertheless, HAV infections still constitute one of the leading causes of outbreaks of foodborne illness, particularly in developing countries [29, 49, 66]. The Centers for Disease Control and Prevention (CDC) recorded 5,683 cases of HAV infection including 14 hepatitis-A-related deaths in the USA in 2004 [13] many of which were believed due to food consumption behaviours. The products that represent a higher risk for contamination are ready-to-eat or minimally processed products that have been in contact with contaminated surfaces or contaminated by an infected person. In addition, the prevalence of viruses on different food surfaces has been attributed to their high stability in the environment; hence the occurrence of HAV infection in developed countries as well. In a food preparation setting, surfaces can be contaminated by food handlers with poor personal hygiene, which can lead to the transfer of the virus to various food products [21]. In developed countries, the group at the greatest risk of infection is international travellers, depending on the living conditions, the length of stay and the incidence of HAV infection in the region visited [44, 47]. Vaccination of travellers to zones where HAV is endemic is, therefore, a highly recommended strategy [47].

**Norovirus**

Since the discovery of norovirus in 1972 after an outbreak in Norwalk, Ohio by A. Z. Kapikian, the discovery of its route of transmission, biology and structure has been pursued [43]. However, the lack of a propagation system for the laboratory setting has hampered the complete understanding of the actual receptors involved in the propagation of this virus. Recent discoveries of surrogates (feline calicivirus and murine norovirus) as well as study models (virus-like particles) have increased the understanding of the basic molecular functions of human norovirus. In addition, the development of information resources such as FoodNet and European surveillance has contributed to knowledge about its epidemiology and economic burden.

Human noroviruses belong to the genus *Caliciviridae* and contain single-stranded positive-sense RNA genomes of approximately 7.5 – 7.7 kb in a 27-nm-diameter virion. These viruses present high genomic variability, which makes them highly adaptable and able to persist in new environments [77]. The norovirus virion encodes three open-reading frames (ORF) and is composed of 90 dimers of the major capsid protein VP1 and one or two copies of the minor structural protein VP2 (Figure 2). Some advances in cell culture of human noroviruses are based on the use of a small intestinal epithelium model as three-dimensional aggregates [87] or transfection of norovirus RNA into human hepatoma cells [28]. A major advance was the discovery of a murine norovirus (MNV-1) that replicates in macrophages of dendritic cells. MNV-1 shares many biochemical and genetic features with human noroviruses such as size (28 to 35 nm in diameter), shape (icosahedral) and buoyant density ($1.36 ± 0.04$ g/cm$^3$) [101]. Moreover, analysis of the MNV-1 genome indicates three ORFs which are characteristic of noroviruses and vesiviruses, two genera within the *Caliciviridae* (Figure 2). Phylogenetic analysis of the viral capsid protein and viral genome corroborates that MNV-1 was a previously unknown norovirus. The broad genetic diversity of noroviruses has allowed strains to adapt to different regions, hence their

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**Figure 2.** Organization of the norovirus genome. The genome has three ORF, ORF1 encodes nonstructural proteins synthesized after the polyprotein is autoprocessed by a 3C-like protease, ORF2 encodes the major structural capsid protein and ORF3 encodes a minor structural protein (adapted from Hardy 2005 and Donaldson et al. 2010).
distribution around the world [56]. It has been suggested that this genetic diversity is the result of recombination among strains, which has complicated the identification and therefore the classification of novel virions [1]. New methods for classification therefore have been proposed using either the entire gene sequences of the major capsid protein or both the RNA polymerase and the capsid gene sequences to provide better phylogenetic insight into these viruses [1, 103]. These classification systems will lead to a better understanding of norovirus activity around the world and have already helped to identify strains belonging to the GI.4 cluster as being the most prevalent in outbreaks worldwide [10, 103]. The contribution to virology of noroviruses with unique biological characteristics has helped to discover new pathways and functions for viral proteins and their interactions with cellular proteins [33].

The development of reverse transcription–polymerase chain reaction (RT-PCR) in the early 1990s shed more light on norovirus infections and these are now believed to be the cause of more than 85% of the outbreaks of viral gastrointestinal illness that occurred between 1995 and 2000, as reported by the European Network [33]. Nevertheless, whether the increased prevalence of illness due to norovirus is the result of improved diagnostics or an actual increase in its burden of illness remains controversial. Factors suggesting that norovirus disease may actually be more common today include i) a general reduction in the incidence of bacterial foodborne diseases, due in large part to initiatives such as improved refrigeration and HACCP systems to decrease food contamination and ii) increased vulnerability to norovirus infection due to changes in lifestyle compared to when these viruses were discovered [100]. In addition, new findings about the prevalence of norovirus among international travellers and asymptomatic individuals suggest other factors that may contribute to the propagation of norovirus [2, 7].

Factors affecting viral persistence on surfaces

Mechanisms of attachment

The attachment mechanisms of pathogens have been studied more comprehensively for plant pathogens and symbionts on plant surfaces. In the case of bacteria, specialized structures such as flagella, fimbriae, extracellular polysaccharides and outer membrane proteins are involved in attachment [91]. The strength of the attachment of bacteria such as Salmonella, E. coli, L. monocytogenes to cantaloupe rinds has been highly correlated with negative and positive surface charges as well as hydrophobicity [91]. Since the viral capsid is composed of protein subunits in repeating structures, hydrophobic, hydrophilic and neutral attractions may all be involved in the adsorption of a virion to a substrate [93]. The attachment of viruses to different surfaces is therefore affected by surface physical and chemical properties as well as specific receptors that may be present. Physicochemical factors are involved mainly in the attachment of viruses to inanimate surfaces, whereas special receptors are involved in the attachment of viruses to live cell epithelial surfaces. Le Guyader and Atmar (2008) described some of the physicochemical factors that affect the attachment of viruses to different food surfaces.

Among those physical factors, the characteristics of the surface play a key role in the adhesion of microorganism to different surfaces. In the case of the microbial adhesion to different surfaces, which has been described more for bacteria; it has been shown that in addition to surface parameters (cleaning procedures, material hydrophobicity, conditioning films), topographical features as well as the shape, size and orientation combined with cell properties greatly influence attachment. This effect can be even stronger than specific attachment for specialized structures (pili, flagella) [98]. One parameter used to quantify the roughness of a surface is the $R_a$ value, which represents the average departure (in micrometers) of the surface profile from a mean centre line [94]. It describes variations in the topography in the vertical direction but does not consider changes in the lateral direction. Some studies have suggested a minimum roughness value below which bacteria are not retained. Therefore, characterization of the surface topography including scratches due to abrasion and pits due to impact damage (both of which provide niches in which microorganisms
are protected from shear surfaces) and hygiene and cleaning measures are important (Figure 3) [94]. A small number of studies have determined some of the particular physicochemical parameters that are involved in the attachment of foodborne viruses. It has been found that fingers can transfer noroviruses sequentially to up to seven clean surfaces. Once the viral particles are transferred via fingers to a surface such as melamine, they can be passed to other typical hand-contact surfaces such as telephone receivers, door handles and taps [6].

The capacity of attachment of different viruses is broader as viruses are able to attach to surfaces of different materials and likely to be involved in transmission of norovirus (flush, tap and toilet, handles, toilet seats, door) [6]. While hand washing might be enough to eliminate viral particles, surfaces must also be decontaminated when an outbreak occurs. The disinfection technique has to be specific for each different surface due to ineffective removal of viral contamination, for example, the use of cloth [6]. Viruses have been found to remain infectious for up to three days on telephone buttons and receivers or for one or two days on telephone wire or computer mouse [16]. Differences in the persistence of viruses have also been observed on food preparation surfaces. Norovirus GI and FCV are able to survive on stainless steel, formica and ceramic surfaces for up to seven days at 22 ± 2 °C and ambient relative humidity [21] whereas norovirus GII might persist for up to 42 days, as demonstrated with snow mountain virus [58]. D’Souza et al. (2006) also observed that norovirus GI and FCV dried on stainless steel were more easily transferred to wet lettuce than to dry lettuce. Moreover, norovirus was transferable to dry lettuce from stainless steel after 10 min of drying and to wet lettuce after 60 min of drying. More recent findings have shown that 10 min is enough for up to 10^3 RTPCRU of norovirus GII or MNV-1 to remain attached to stainless steel at pH 4, 7 or 9 at either 25 or 80% relative humidity [25]. Norovirus GI appears to remain infectious for at least two hours on hands [58]. The results of this study are important since epidemiological data suggest a higher incidence of norovirus outbreaks due to strain GII. Some studies show that viral transfer is easier if both inoculum and surface are wet, possible due to facilitation by moisture. The association of human norovirus with water may explain why this virus remains infectious on surfaces for longer than other viruses such as astrovirus, poliovirus and adenovirus under ambient conditions and might explain their more frequent involvement in food-related outbreaks of gastrointestinal illness.

The attachment of viruses to specific receptors has shown to be more complex and to date these receptors remain unknown. Some strains or model particles adapted to laboratory conditions have provided some insight into possible sites involved in this specific interaction. Since norovirus is one of the most difficult pathogens to propagate, two different in vitro systems have been suggested, one on an epithelial cell line and the other in a three-dimensional system. A system using Caco-2 cells and virus-like particles (VLP) expressing the norovirus VP1 capsid protein to mimic the initial steps of norovirus infection in the human intestine has been proposed recently [69]. Although low, the increased frequency of VLP attachment to cells cultured for longer periods (10 days) suggested that norovirus attaches preferentially to differentiated cells. This study also revealed negative effects of bovine colostrum on the attachment of VLPs to Caco-2 cells, suggesting that possible defensive factors could be used in

Figure 3. Schematic representation of physical entrapment of viral particles in different types of crevices that could be present on a food contact surface. In the case of food products, smooth surfaces would present few peaks or grooves (A) as on an apple, rough surfaces with few valleys can be observed on oranges and avocados (B) and very rough food surfaces with big cavities characterize a cantaloupe (C) (adapted from Wang et al. 2009).
food. The ability of different viral particles to attach to cell receptors has been associated with conformational changes in the capsid induced by receptor binding. For example, feline junctional adhesion molecule (fJAM-1) has been shown to induce changes in capsid conformation that appear to precede the un-coating of the FCV genome. This adhesion molecule was recently identified as the first functional receptor for the *Caliciviridae* family. Its role may be to cause the formation of a pore through which the viral genome is released into the cell [8]. Histo-blood-group antigens (HBGA) have been associated with the attachment and genomic variation of the norovirus genogroup. HBGA have been suggested as receptors or co-receptors for norovirus infection, although no direct observation of HBGA binding mediating viral entry into cells has been documented. Some norovirus strains such as GI.4 2004, GI.4 2005, GI.3b and MNV1 do not appear to bind to artificial HBGA, suggesting that other receptors might be involved in infection [22]. Recognition of different ligands has therefore been suggested for specific binding. Recognition of A-like ligands might result in virus persistence whereas release or quicker degradation might be observed when a sialylated ligand is involved. The differences in the prevalence of GI.4 and GI.1 in oyster tissues have been attributed theoretically to different ligands as well as nonspecific binding [60]. In contrast, VLP expression has provided more information on the binding of VP1 to specific carbohydrate ligands. These studies have demonstrated that different norovirus genotypes may recognize specific type-1 and type-2 carbohydrate ligands, which may lead to different noroviruses targeting different tissues and therefore withstanding the human immune system and persisting in the food chain [22]. The role of carbohydrate ligands in increasing the attachment of enterovirus has also been observed. Jonsson et al. [42] demonstrated that attachment of enterovirus to cells was greater for crude viral extract than for virions purified by ultracentrifugation and suggested that gradient purification altered virion structure or removed components required for interaction with the cell surface. Studies of this type might help to determine the role of attachment in the persistence of foodborne viruses on different food or agri-food surfaces under actual conditions of disease spread.

While some efforts have been directed towards uncovering how viruses attach to different surfaces, others have focused on microscopic observation of viral particles bound to food or environmental surfaces. Kukavica-Ibrulj et al. [51] used anti-HAV-specific antibodies and confocal microscopy to observe the rapid attachment of HAV to stainless steel, copper, polythene and polyvinyl chloride (PVC) at 20°C and 4°C. Rawsthorne et al. [78] described a fluorescent *in situ* method of observing HAV particles on onion epidermis. Biotinylated viral particles were linked to streptavidin-coated Q-dots (655) prior to inoculation to onion epidermis. Visualization by confocal microcopy of viral particles after rinsing with beef extract buffer suggested that attachment of HAV was not only by electrostatic interaction but also by a more specific binding not disrupted by competition with glycine or other buffer components. Observation of particles remaining bound to the onion surface raises the question of the role of the buffer used to elute virus from food surfaces in most of the detection methods described in the literature. This assay could be used to observe other viruses such as human norovirus or rotavirus on other produce and to investigate the prevalence of certain strains in the food chain. Immunohistochemistry has also been suggested for the on-site study of the viral attachment. A recent elaborate immunochemical method allowed the observation of norovirus in the digestive tract, gill and labial palp tissues of Pacific oysters fed 80 PCR amplifiable units per ml of seawater for 48 hours, indicating that the localization of viral particles in oyster tissue may be similar for a number of different virus types [65]. Using confocal microscopy and SYBR gold stock solution, Wei et al. [97] were able to demonstrate that murine norovirus is able to infiltrate surface crevices of lettuce. Viral particles were observed in the stomata of lettuce leaves after rinsing with Virkon. Higher levels of internalization were observed for viral particles suspended in bio-solids rather than in manure prior to inoculation to the lettuce. These findings strongly support the hypothesis that viruses attach...
not only by electrostatic forces but also by more specific interaction with certain receptors on the food surface. The identification of the protruding domain of the viral capsid protein as the attachment site for HBGA via a conformational pocket seems to indicate that the interaction of the viral capsid with individual oligosaccharide residues might be involved in the binding of viruses on different food surfaces [88]. Furthermore, receptor-binding sites have been shown to differ among norovirus strains, in terms of location and receptor-binding process as well as amino acid composition and hence structure. This strongly suggests that carbohydrate receptor recognition may be the specific interaction in foods other than shellfish.

**Influence of food matrix**

As discussed above, the type of interaction between viral particles and a food depends on the composition of that food surface. For bacteria, intrinsic factors such as cell wall proteins, adhesins, cell envelopes and extracellular polymers have a greater effect on their adherence to different inanimate surfaces than does the composition of the surface itself [89]. In the case of viruses, capsid composition and size both affect adherence, since viral particles can lodge in microscopic pits or scratches on the contact surface. The presence of inactivating compounds such as disinfectant also affects the persistence of viruses in the environment. Among the factors that affect the binding of viruses to different food surfaces (Figure 4) are the properties of the virus (e.g. its isoelectric point), pH and ionic strength of the solution, characteristics of the sorbent and the presence of compounds competing for sorption sites [55]. Since norovirus cannot be cultured in the laboratory, little is known about the length of time it remains infectious in the environment or the effectiveness of disinfection procedures used to inactivate it. Surrogates and other non-enveloped viruses have been studied to estimate the persistence of the infectiveness of norovirus and perhaps other viruses. Another alternative for studying the persistence of noroviruses in food is the synthesis of virus-like particles. VLPs are synthesized by a baculovirus system that expresses one or more capsid proteins. Using this type of surrogate, Loisy et al. [59] determined the persistence of rotavirus-like particles in oysters after depuration, a dynamic process used to eliminate contaminants from shellfish either in land-based facilities or natural settings. When shellfish are transferred to a clean growing area, the depuration process is called relying. Differences in VLP elimination time were found by RT-PCR, depending on the initial particle load. Loads of $10^5$ VLP/oyster were eliminated after 37 days, while $>10^4$ VLP/oyster were reduced to undetectable levels after 82 days of relying. An initial 7-day depuration step did not produce a significant decrease and VLP elimination was more effective with relying. More recent studies have suggested that eliminating viruses from shellfish depends on the nature of the virus. In the case of HAV, six weeks of depuration did not eliminate viral particles in oysters, although no infectious particles were isolated after four weeks [45]. Ueki et al. [90] showed that high

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**Figure 4. Factors that affect the persistence of viruses on agri-food surfaces.**
concentrations of FCV (2.2 x 10^2 ± 3 x 10^3 copies/g) could be eliminated from oysters after a 10-day relying process. In contrast, the concentration of norovirus GI.1 (1.8 x 10^3 copies/g) during the same process was not significantly affected. The difference between HAV and VLP elimination time in oysters might have been due to the different nature of the viral particles and the temperature at which the studies were carried out. In the HAV study, the seawater temperature was set at 10 ± 2 °C while VLP depuration was done at 19ºC and 22ºC with a salinity of 33% to 34.5%. Water temperature has recently been identified as a factor contributing to viral accumulation in oysters, together with gonadal development, glycogen content in the connective tissue and mucus production [60]. Different environmental conditions that contribute to the persistence of viruses on different surfaces are discussed in the following section. Variation in the persistence of different strains of norovirus in mussels in an effluent plume has also been observed. Norovirus GI.1 was detected in 45% of blue mussels (Mytilus edulis) collected, while other norovirus strains (GI.2 and GI.4) were detected in only 5% of the samples [71]. Water temperature and salinity were not reported but the higher frequency of norovirus GI than norovirus GI in water and the bivalves suggested a higher environmental stability, since 100% sequence similarity was found in samples from patients infected three months before mussel sampling. It is now widely accepted that receptors similar to human intestinal receptors are likely involved in the attachment of viruses to shellfish tissues, hence the long persistence of viruses in this type of food even after depuration treatments. This receptor interaction might not be only with norovirus genogroup II but also with HAV since both viruses have been found in clam tissues [46]. HAV has remained in an infectious state for up to four weeks in marinated mussels at 4ºC after a treatment of 37 s in boiling water followed by steaming for 3 min and 8 s [35]. It was suggested that norovirus might remain infectious for as long following such treatment since changes in titer were not detected using molecular methods. However, the lack of a cell culture method for norovirus quantification hampers the determination of its infectivity in persistence studies. Differences in the attachment of norovirus genotypes to different shellfish tissues have been demonstrated recently [61]. GI.4 in oysters was not restricted to digestive tissue but was also found in gills, while GI.1 was found to accumulate only in the digestive tissues. Manso et al. [63] detected one serotype of HAV in different shellfish. Sixty-nine strains of HAV belonging to serotype IB were detected at concentrations of 3.1 x 10^2 to 1.4 x 10^10 RNA copies/g of clam, mussel and cockle digestive tissue. Polo et al. [76] also reported high levels of HAV and two norovirus genotypes in contaminated imported shellfish. Noroviruses GI and GII as well as HAV contamination levels in the range of 4.7 x 10^3 to 7.7 x 10^7 RNA copies/g of digestive tissue were detected in molluscs from Morocco, Peru, Vietnam and South Korea. Adenovirus has been suggested as a viral indicator in bivalves due to its ability to remain in shellfish for up to 10 weeks [34]. Adenovirus accumulation was more evident in gills and glands of oysters and mussels at 4ºC. Although detection of adenovirus was possible for up to 42 days at 4ºC and 18ºC, cytopathic effects showing infectivity were observed for up to 7 days at 4ºC and 14 days at 18ºC. All of these studies corroborate the highly stable interaction between the viral capsid and receptors in shellfish, which allows the virus to persist in this type of seafood at different salinity and temperatures. Further study of the persistence of noroviruses as infectious agents in shellfish at different water temperatures is therefore justified.

The specific interaction between virus and receptors in shellfish also determines the localization of different viral particles and therefore the accumulation in different tissues. In general, assays of virus titer have focused on the stomach and digestive diverticula. The accumulation of viruses in oysters has been found lower in these organs than in the gills, which are now suggested as a new target tissue for virus detection [95]. The type of shellfish has also been found to influence the accumulation of different viral strains. In blue mussels, a higher affinity for norovirus GI.4 compared to GI.3b has been observed [17], while sapovirus was found in greater numbers in clams (Corbicula japonica) in December 2005 and September 2006 in
Japan [30]. A more recent report about the prevalence of viruses in shellfish (mussels, oysters, cockles and pipi) collected at 28 locations in New Zealand showed variable numbers of norovirus, enterovirus and adenovirus, with noroviruses and adenoviruses detected in about 30% of samples and enteroviruses detected in 17% (more often during the winter months) from January 2004 to February 2006. From October 2007 to September 2008, 32% of samples were positive for norovirus, norovirus GI being less frequent than norovirus GII strains [26].

The few studies on the persistence of foodborne viruses suggest that the interaction between the viral particle and food surfaces other than shellfish is less specific. For example, the primary force involved in the adsorption of feline calicivirus and bacteriophages \( \varphi X174 \) and MS2 to butterhead lettuce was electrostatic [93]. Nevertheless, the period of time for which viruses remain on food seems not to be affected. Such is the case of MNV-1 on frozen onions and spinach, where this virus was detected within six months after blanching before deep-freezing of shredded onion and chopped spinach. However, the number of MNV-1 among the storage months was variable during the six months [5]. More than \( 10^5 \) PFU MNV-1 were transferred to both spinach and onion bulbs from wash water loaded with about 4.8 log PFU/ml. Infectious MNV-1 was counted in potable water and levels remained constant for one week of storage at room temperature. This study also determined that MNV-1 seemed more resistant to inactivation by peracetic acid [4]. Other viruses that resist for long periods of time at freezing temperatures are HAV, norovirus GI or GII, FCV and rotavirus. These viruses remain infectious on frozen strawberries, raspberries, blueberries, parsley and basil (-20°C) for up to 90 days with reductions of less than 1 \( \log_{10} \) [11]. The findings of this study showed that norovirus GII is more sensitive than GI to freezing, since a 2.3 \( \log_{10} \) reduction was observed after 90 days on blueberries. The persistence of FCV at refrigeration temperatures has been observed for up to 7 days on lettuce, strawberry, ham and stainless steel being ham the most favourable matrix for the survival of this norovirus surrogate [64]. Lamhoujeb et al. [52] determined that infectious norovirus particles were able to persist on turkey slices and on lettuce at 7°C for over 10 days. Norovirus eluted from both types of food surfaces were treated using an enzymatic procedure to differentiate between active and inactive particles by real-time NASBA. Interestingly, the reduction in the norovirus titer on lettuce over the 10-day period was essentially linear, while no significant difference in titer was found on turkey slices. Differences in persistence on different food surfaces have been attributed to surface morphology, smooth surfaces such as lettuce appearing to provide less protection than rough ones such as turkey or ham. Moreover, food components such as proteins and fat seem to help to stabilize the virion. This protective effect of the food surface has also been observed in inactivation studies. Inactivation of FCV, AiV and HAV by UV light has been found greater on lettuce than on strawberries and attributed to the topography of both food surfaces [23]. Viral particles can be sheltered or hidden from UV light on the surface of a strawberry but are more exposed on lettuce due to the smoothness of the surface. Nevertheless, another factor to take into account is the fine structure of the food surface since internalization of bacteria in cut edges, trichomes or stomata has been previously described [81]. Internalization of Salmonella in stomata opened by exposure to light has been observed, while lettuce leaves kept in the dark do not present as high a concentration of Salmonella [50]. Similar results were obtained in a study conducted by Urbanucci et al. [92] in which norovirus and canine calicivirus were internalized at low concentrations in lettuce roots. Moreover, canine calicivirus was detected by real-time PCR in lettuce leaves. The determination of light-induced internalization of viruses should take into consideration that compounds potentially secreted by the plant might inhibit this process and/or the attachment of the virus to the phyllosphere. The infectivity of the internalized particles should also be determined in order to assess the potential risks for consumers of fresh produce. In addition, the characterization of food surface topographies may provide better understanding of viral entrapment or attachment, as it has recently for other foodborne pathogens [83, 96].
Importance of environmental conditions

As demonstrated in several studies, the persistence of viruses under different environmental conditions depends on the mode of transmission to different food-contact surfaces. As mentioned in previous sections, the factors that influence the spread of a foodborne pathogen are the concentration and frequency of the infectious agent, the concentration deposited in food, the temperature to which the pathogen has been exposed and its capacity for staying in an infectious state in the environment and on hands [89]. Although the presence of viruses in water has been confirmed in numerous studies, focus on the persistence of the viruses most common in food is quite recent.

The following are some of the factors that have been found to affect significantly the persistence of waterborne viruses [38]:

- Total organic carbon from 6.0 to 7.8
- Hardness from 29 to 339 mg CaCO$_3$
- Turbidity from 2.5 to 36 NTU
- pH in the range of 6.0 to 7.8
- Temperature from 4 to 37 ºC.

These parameters might not apply for viruses in a food surface since disinfection, different temperatures and preservation processes may be involved. Cheong et al. [15] have shown that virions present in water used for irrigation of vegetables can be identified later in the produce. Viruses such as adenovirus and enteroviruses have been found in ground water and norovirus has been found together with adenovirus in spinach. Persistence of infectious MNV in stool suspension (1:1) may be up to 40 days at 4ºC, whereas losses occur at 18°C and 30ºC after only 24 h [54]. High-temperature-short-time conditions also affect the stability of MNV, 60ºC for as little as 2.5 min reducing viral titer by the same extent as observed for norovirus [4]. In comparison, 0.5 M or 1 M NaCl for 72 h has little impact on MNV. It has been suggested therefore that the persistence of norovirus in the environment is greater at low temperatures, which might explain the higher incidence of norovirus outbreaks during winter than in the summer [54].

Moreover, the persistence of MNV at low salt concentrations matches the persistence of norovirus in seawater and consequently in
shellfish [54]. Longer persistence of noroviruses and enteroviruses at 4°C than at 20°C in natural wastewater biofilms has also been observed [85]. Although viral load did not vary significantly among monthly samplings during a two-year study in a moving-bed biofilm reactor, a seasonal prevalence of norovirus GI during winter was observed. In contrast, no significant variations of norovirus GII and enteroviruses were observed from winter to summer. A dynamic equilibrium between viral particles protected from degradation when associated with solids and viral particles released due to physicochemical changes in the water (pH, temperature, exposure to UV light) was hypothesized. A one-year study of tap water showed that norovirus GI and GII was able to persist at temperatures of 14-24°C, norovirus GII showing higher prevalence but not detected around 25°C and pH 8.0 [31]. Subsequent findings showed that norovirus GI and GII are more prevalent during winter than during summer in river water [32]. The temperature at the sampling sites varied from 2 to 22°C, showing that both noroviruses persisted at lower temperatures, as they do in seawater.

The effect of humidity on persistence on produce depends on the type of virus. In the case of HAV, lower inactivation rates (0.01/day) have been observed under dry conditions (45-48% RH) than in high humidity (85-90% RH) on cantaloupe, lettuce and bell peppers at 25°C [86]. Inactivation of FCV is much faster (up to 1.13/day) under the same conditions and on the same produce [86]. FCV is thus more sensitive to environmental conditions (pH, temperature, relative humidity) than MNV-1, even though both viruses are more resistant under cool (4°C) and damp conditions [12]. MNV-1 in suspension is able to persist for seven days at 4°C without significant reduction, while loss of FCV is significant under the same conditions. MNV-1 appears more resistant to pH 2 or 10 and more stable at pH 6-9, while resistance at 63 and 72°C is similar to that of FCV. In comparison, norovirus lasts for two months in mineral water and tap water at -20, 4 and 25°C, but is likely to last only half as long in river water or sewage at 4°C or 10 days at 25°C. This is in contrast with more recent findings concerning the persistence of norovirus in water (> 80 days) [72].

The greater stability of foodborne viruses such as norovirus at low temperatures has prompted some researchers to examine the stability of norovirus-like particles as norovirus surrogates. The high stability of the norovirus virion has been corroborated by the high stability of norovirus VLPs at temperatures of up to 55°C at pH 3-7 [3]. Temperatures above 60°C and pH above 8 induce changes in the secondary, tertiary and quaternary structures of VLPs, which is consistent with loss of viral infectivity at higher temperatures [3, 19]. Since norovirus is quantified using mainly molecular methods, which detect infectious and non-infectious particles indifferently, some way of determining infectivity after different treatments will have to be proposed.

Similar to the findings for foodborne viruses in water, higher temperatures have been shown to destabilize the viral capsid and thus affect the persistence of viruses in different food matrices. Common processing temperatures such as 63°C and 72°C can affect significantly the infectivity of HAV and MNV, while a less significant reduction in the qRT-PCR titer of norovirus GI and GII has been observed [36]. Norovirus GI seems to be more resistant than norovirus GII to heat treatment in solution [36]. These determinations were done in liquid matrices (water and milk) and the effects in solid matrices remain to be determined. Moreover, effects on the viral capsid should be determined in order to detect changes that could have a negative impact on virion integrity and hence infectivity.

**Role of inactivation processes**

Persistence of viruses is also dependent on the integrity of the capsid. The capsid protects the viral genome against damage due to environmental factors such as pH, temperature and light. Moreover, recognition of a receptor for entry of the virion into a host cell occurs through contact with the capsid. The capsid also determines the interaction of the virus with the host cell receptors. The capsid and the genome must therefore both function in order to ensure propagation and consequently the continued existence of the virus. The inactivation of foodborne viruses has become preoccupying in recent years due to emerging food processing
technologies. Increasing consumer demand for products that are minimally processed but safe has focused trends towards nonthermal processing technologies, which provide a convenient product with a fresh taste and improved nutritional value. Processes for the inactivation of the most common foodborne viruses have been reviewed comprehensively [27, 37]. In this section, we focus on recent efforts directed towards inactivating viruses in food.

High-pressure processing is a nonthermal process in which very high pressures are applied to foods preserving nutritional value and fresher taste as well as better appearance and texture than can be obtained using conventional processes. A variety of HPP food products have been commercialized [74], but the effect of this treatment on enteric viruses is still under study. The parameters that influence the success of a HPP process include the pressure range, temperature, pH, solvent composition, and in the case of viruses, the structure of the protein as well. Different hypotheses have been proposed regarding the mechanisms of viral inactivation by HPP, based on the proteinaceous nature of the viral capsid, such as extrusion of nucleic acid from the viral particle or changes in the capsid or receptor recognition proteins [37]. Nevertheless, recent studies have not shown significant effects of HPP on viral reduction in food products or in viral suspensions. Sharma et al. [82] showed a small reduction in HAV or FCV on sausages processed at 500 MPa for 5 min at 4°C. These results were unexpected in view of similar reductions of 2.89 and 3.23 log cycles respectively for FCV and HAV obtained with HPP and hydrodynamic pressure processing. More recently, a study by Black et al. [9] showed low inactivation rates of HAV and coliphage T₄ at 250 and 500 MPa in PBS, artificial seawater and oyster slurry. Complete HAV inactivation was possible in PBS and oyster slurry at 500 MPa within 1 min and in artificial seawater for 10 min at the same pressure. The authors suggested stabilization of the capsid protein conformations at the different pressures to explain the mild or reversible effects on the suspended viral particles. These findings led to investigation of whether or not high-pressure treatment causes conformational changes at the molecular level in the viral capsid and whether the protein conformation is stabilized or undergoes changes that do not affect viral attachment to receptors or propagation in vitro, consequently exposing consumers to contaminated product.

Differences have been observed in the prevalence of viruses on the surface of different foods treated by different inactivation processes. UV is effective at damaging the viral capsid and genome and hence reducing infectivity. The effects of UV light vary according to the food surface, for example reduction of viruses such as HAV, FCV and aichi virus is complete on smooth surfaces such as lettuce, while the reduction on strawberry is partial [23]. At higher doses (> 1000 mWs/cm²), UV light can affect the capsid enough to make the genome vulnerable to RNase present in the environment. Other technological processes such as acidification, cooling, freezing and pasteurization have proven ineffective for inactivating norovirus within a food matrix or on a food surface [68]. In this sense, emerging technologies such as pulsed UV light has demonstrated to be an alternative to inactivate viruses on food contact surfaces or in drinking water [39]. Total inactivation of MNV-1 and HAV on stainless steel or PVC is possible using a fluence of 0.060 W s/cm² of pulsed UV light [39]. The efficacy of the treatment was diminished by the presence of particles such as proteins due to protective effects but 3-log reductions were obtained using low fluences. Therefore, protective effects of food components must be taken into consideration for viral inactivation, since norovirus is able to persist in commodities such as noodle salad (24 days at 6°C, pH 5.0-5.5), tomato ketchup (58 days at 6°C, pH 4.5), apples (7 days at 11°C), lettuce (5 days at 11°C), mincemeat (8 days at -18°C, 2 days at 6°C, 30 min of boiling or 30 min at 200°C) and frozen pizza (14 days at -18°C) until consumption or the limit of product storability [68].

Chemical inactivation has also been suggested for reducing the persistence of viruses in the food industry and in clinical environments, in particular on surfaces. Sodium hypochlorite has been demonstrated to be highly effective for inactivation of HAV after 5 min contact at a 12% concentration [40] or norovirus at 3% after 10 min
contact [25]. Sodium bicarbonate has also proven to be a rapid virucidal agent, 5% solution or 1-2.5% with 1.3% glutaraldehyde or 2% with 2% hydrogen peroxide inactivated FCV within 1 min of contact [62]. Since it is now widely accepted that molecular detection of viral particles and biological assay of viral infectivity are poorly correlated, some of the data obtained must be re-examined. For example, MNV-1 may be heat-inactivated based on plaque assay but is still detectable by real-time RT-PCR [4]. Pre-treating the samples with proteinase K and then RNase to degrade any RNA thus released from heat-damaged virions was therefore suggested as a way of eliminating these from RT-PCR analysis. However, this procedure has shown not to improve the correlation between molecular detection and plaque assay and differentiation of infectious particles has not been possible by the molecular procedure.

CONCLUSIONS

The aim of this review is to highlight the scarcity of information on the elements that enable viral particles to remain on food surfaces for long periods of time. The effect of attachment to solid surfaces appears to depend on virus type, notably increasing HAV and norovirus persistence. The factors that determine persistence need to be investigated in greater depth not only for the more common viruses found in food but for other emerging viruses as well. The diversity of food matrices and novel processes make this task more challenging. In addition, the introduction of exotic and ethnic foods into a particular market will certainly make the task more challenging for technologies currently under development. Nevertheless, the benefits to public health are unquestionable in terms of design of appropriate intervention strategies. Finally, the increased availability and affordable cost of microscopy technologies such as confocal laser scanning microscopy will definitely play a significant role in improving understanding of the interactions between viruses and foods and agri-food surfaces.

REFERENCES