CHAPTER 6

Survival and Transport of Enteric Viruses in the Environment

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1.0. VIRUSES IN THE ENVIRONMENT

1.1. Viruses and Environmental Virology

Environmental virology may be defined as the study of viruses that can be transmitted through various environments (water, sewage, soil, air, or surfaces) or food and persist enough in these vehicles to represent a health threat. A wide variety of different viruses, representing most of the families of animal viruses, can be present in human and animal fecal wastes and urine. Especially important are a variety of nonenveloped human and animal enteric pathogenic viruses that can enter the environment through the discharge of waste materials from infected individuals; contaminate food products and drinking and recreational waters; and be transmitted back to susceptible individuals to continue the cycle of infection (Table 6.1). It is estimated that billions of cases of gastrointestinal illness occur annually worldwide (Parashar et al., 1998; Oh et al., 2003). A good deal of these diarrheal cases are to some extent the result of fecal contamination of the environment (Cabelli et al., 1982; Koopman et al., 1982; Fattal and Shuval, 1989; Moore et al., 1994) while outbreaks of hepatitis A and E are associated with water, shellfish, and crops (Melnick, 1957; Reid and Robinson, 1987; Halliday et al., 1991; Bosch et al., 1991, 2001).

The significance to human health of many of the non-human animal viruses present in environmental samples is less well understood and remains uncertain or unknown for many of them. It is remarkable, however, that zoonotic viruses infecting humans continue to be discovered or appear to reemerge as important human pathogens. One example of an emerging disease is severe acute respiratory syndrome, or SARS, reported in November 2002 (Ksiazek et al., 2003). The primary mode of transmission of the SARS coronavirus appears to be direct mucous membrane contact with infectious respiratory droplets and/or through exposure to fomites. Several coronaviruses are known to spread by the fecal-oral route, but there is no current evidence that this mode of transmission plays a key role in the transmission of SARS, although there is a considerable shedding of the virus in stools (Tsang, 2003).

As a scientific discipline, environmental virology was born after a large hepatitis outbreak occurred in New Delhi between December 1955 and January 1956. The origin of the outbreak, which was attributed to hepatitis A at the time but now confirmed to be hepatitis E, was the contamination

Table 6.1 Human Enteric Viruses with Potential Environmental Transmission

Genus	*Popular Name*	*Disease Caused*
Enterovirus	Polio	Paralysis, meningitis, fever
	Coxsackie A, B	Herpangina, meningitis, fever, respiratory disease, hand-foot-and-mouth disease, myocarditis, heart anomalies, rash, pleurodynia, diabetes?
	Echo	Meningitis, fever, respiratory disease, rash, gastroenteritis
Hepatovirus	Hepatitis A	Hepatitis
Reovirus	Human reovirus	Unknown
Rotavirus	Human rotavirus	Gastroenteritis
Mastadenovirus	Human adenovirus	Gastroenteritis, respiratory disease, conjunctivitis
Norovirus	Norwalk-like virus	Gastroenteritis
Sapovirus	Sapporo-like virus	Gastroenteritis
Hepervirus	Hepatitis E	Hepatitis
Mamastrovirus	Human astrovirus	Gastroenteritis
Parvovirus	Human parvovirus	Gastroenteritis
Coronavirus	Human coronavirus	Gastroenteritis, respiratory disease
Torovirus	Human torovirus	Gastroenteritis

by sewage, from 1 to 6 weeks prior to the epidemic, of the Jumna River, the source of water for the treatment plant. Alum and chlorine treatment prevented bacterial infections, but 30,000 cases of hepatitis occurred among the population. As a consequence of this outbreak, studies in water and environmental virology began with efforts to detect poliovirus in water around 50 years ago. Since that time, other enteric viruses responsible for gastroenteritis and hepatitis have replaced enteroviruses as the main target for detection in the environment, although the near eradication of poliomyelitis from the globe calls for exhaustive studies on the occurrence of wild-type and vaccinal-type polioviruses in environmental samples.

1.2. Waterborne Transmission of Enteric Viruses

Figure 6.1 illustrates the possible routes of waterborne transmission of enteric viruses. Viruses can be transmitted by a variety of routes, including direct and indirect contact, vector transmission, and vehicle transmission. Viruses are shed in extremely high numbers in the feces of infected individuals; patients suffering from diarrhea or hepatitis may excrete from 10^5 to 10^{11} virus particles per gram of stool (Farthing, 1989). Furthermore, a single episode of vomit of a patient with norovirus gastroenteritis may contain around 10^7 particles (Cheesbrough et al., 1997). Ingestion of sewage-contaminated water or food is the main route of infection with human

enteric viruses, although the role of inanimate surfaces serving as vehicles for virus infection must not be underestimated. Viruses with a viremic phase, such as the hepatitis viruses, may also be parenterally transmitted, although these days it is considered to be a much less frequent mode of transmission.

A poorly understood aspect in the epidemiology of several enteric viruses is the role of animal viruses in human disease. Nucleotide sequence analysis of some human enteric viruses has indicated a high degree of sequence similarity with animal strains. Notably, hepatitis E virus–related sequences have been detected in pigs (Meng et al., 1997; van der Poel et al., 2001; Banks et al., 2004) and birds (Huang et al., 2002). The threat of zoonotic infections may be either through direct transmission, suspected for hepatitis E virus (HEV; Reyes, 1993) and caliciviruses (Humphrey et al., 1984), or through incidental coinfection of a host with animal and human viruses, resulting in the mixing of genes and generation of novel variants (recombination/reassortment; Unicomb et al., 1999). Recombination has been demonstrated as a mechanism for rapid expansion of diversity for noroviruses and rotaviruses, but it is likely to be a common feature of the RNA viruses involved (Jiang et al., 1999; Unicomb et al., 1999). Viruses related to the human rotaviruses, astroviruses, noroviruses, sapoviruses, and HEV circulate in several animal species, providing a huge reservoir for virus diversity (Shirai et al., 1985; Meng et al., 1997; van der Poel et al., 2001; Huang et al., 2002).

In the water environment, the fate of microbial enteric pathogens may take several potential routes (Fig. 6.2). Mankind is exposed to waterborne

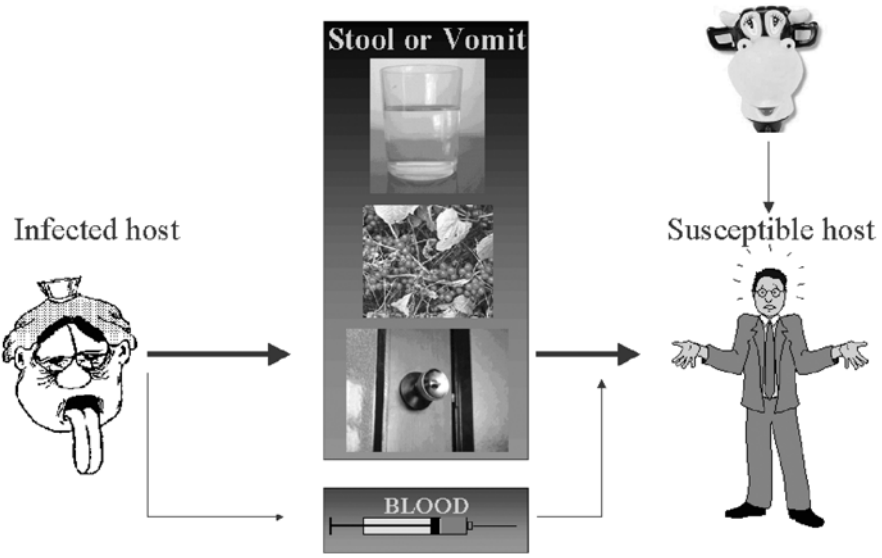

Figure 6.1 Routes of enteric virus transmission. Thick and thin arrows depict the main and minor routes of virus transmission, respectively.

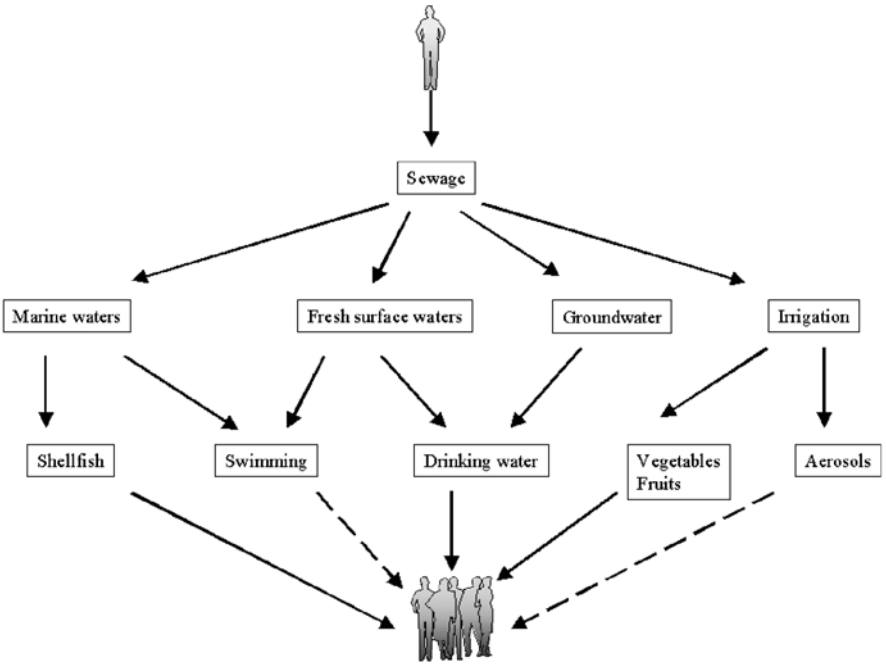

Figure 6.2 Waterborne transmission of enteric virus infections. Dashed lines depict unconfirmed transmission.

enteric virus infections through shellfish grown in contaminated waters, contaminated drinking water, food crops grown in land irrigated with wastewater and/or fertilized with sewage, and, to a lesser extent, sewage-polluted recreational waters (Tables 6.2 and 6.3).

Studies have documented the presence of enteric viruses in raw and treated drinking water (Keswick et al., 1984), and they are also frequently isolated from freshwater environments (Table 6.4). However, epidemiological proof of human infection caused by these viruses as a result of water consumption is scarce. Water-system deficiencies that caused or contributed to these outbreaks may be categorized under five major headings: (a) use of contaminated, untreated surface water; (b) use of contaminated, untreated groundwater; (c) inadequate or interrupted treatment; (d) distribution network problems; and (e) miscellaneous.

Pathogenic viruses are routinely introduced into the environment through the discharge of treated and untreated wastes, as current treatment practices are unable to provide virus-free wastewater effluents. Virus concentrations of 5,000 to 100,000 pfu/L are commonly reported in raw sewage (Rao and Melnick, 1986) and may be greatly reduced during treatment; however an average of 50 to 100 pfu/L are normally found in effluents from wastewater treatment plants (Rao and Melnick, 1986).

Table 6.2 Examples of Waterborne Viral Disease Outbreaks

Type of Water Implicated	*Virus*	*Disease*	*Reference*
Drinking water	Polio	Poliomyelitis	Mosley, 1967; Lippy and Waltrip, 1984
	Echo	Meningitis	Cliver, 1984; Amvrosieva et al., 2001
	Rotavirus	Gastroenteritis	Murphy et al., 1983; Hopkins et al., 1984; Hung et al., 1984; Craun et al., 2002; Villena et al., 2003
	Norovirus	Gastroenteritis	Kaplan et al., 1982; Blacklow and Cukor, 1982; Kukkula et al., 1999; Craun et al., 2002
	Adenovirus	Gastroenteritis	Murphy et al., 1983
	Hepatitis A	Hepatitis	Craun, 1988; Bosch et al., 1991
	Hepatitis E	Hepatitis	Khuroo, 1980; Ramalingaswami and Purcell, 1988
	Parvovirus	Gastroenteritis	Lippy and Waltrip, 1984
Recreational seawater	Rotavirus	Gastroenteritis	Fattal and Shuval, 1989
	Adenovirus		Foy et al., 1968; D'Angelo et al., 1979
	Hepatitis A	Hepatitis	Birch and Gust, 1989
Recreational freshwater	Coxsackie		Cabelli, 1983
	Enterovirus	Gastroenteritis	Lenaway et al., 1989
	Rotavirus	Gastroenteritis	Andersson and Stenström, 1987
	Norovirus	Gastroenteritis	Koopman et al., 1982
	Hepatitis A	Hepatitis	Bryan et al., 1974

Table 6.3 Examples of Large Outbreaks (Over 100 Cases) Linked to Shellfish Consumption

Year	*Country*	*Shellfish*	*No. of Cases*	*Responsible Virus*	*Reference*
1976–1977	Great Britain	Clams	800	SRSV	Appleton and Pereira, 1977
1978	Australia	Oysters	2,000	NoV	Murphy et al., 1979
1978	Australia	Oysters	150	NoV	Linco and Grohmann, 1980
1980–1981	Great Britain	Cockles	424	NoV	O'Mahony et al., 1983
1982	USA	Oysters	472	NoV	Richards, 1985
1983	Great Britain	Oysters	181	SRSV	Gill et al., 1983
1983	Malaysia	Cockles	322	HAV	Goh et al., 1984
1986	USA	Clams Oysters	813 204	NoV	Morse et al., 1986
1988	China	Clams	292, 301	HAV	Halliday et al., 1991
1999	Spain	Clams	183	HAV	Bosch et al., 2001

SRSV, small round structured viruses; NV, Norovirus; HAV, hepatitis A virus.

Table 6.4 Examples of Human Enteric Virus Isolations from Freshwater

River	*Virus Type*	*MPNCU/Liter*	*Reference*
Loire (France)	Enteroviruses and Adenoviruses	1.39	Le Bris et al., 1983
Ripoll (Spain)	Enteroviruses	15.5	Bosch et al., 1986
Besos (Spain)	Enteroviruses	16.2	Bosch et al., 1986
Tiber (Italy)	Hepatitis A virus	+[a]	Divizia et al., 1989a
Undetermined rivers (Germany)	Enteroviruses	0.5 to 56	Walter et al., 1989
Saint-Lawrence (Canada)	Culturable enteric viruses[b]	0.1 to 29	Payment et al., 2000

MPNCU, most probable number of cytopathic units of virus.
[a] Molecular detection of viral RNA.
[b] Unidentified virus grown in MA104 cells and reacting with human immunoglobulin.

Sewage sludge, a by-product of wastewater treatment, is a complex mixture of solids of biological and mineral origin that is removed from wastewater in sewage treatment plants. The sewage may undergo primary treatment (physical sedimentation or settling); secondary treatment (primary sedimentation plus high-rate biological processes, such as trickling filter/activated sludge); secondary treatment plus disinfection (chlorination, peracetic acid, UV or ozone); tertiary treatment (advanced wastewater treatment, including primary sedimentation, secondary treatment plus, for example, coagulation–sand filtration, UV, microfiltration); tertiary treatment plus disinfection; and lagooning (low-rate biological treatment). In any case, the type of treatment will determine the concentration of pathogens in a wastewater effluent and the relative risk of its disposal.

An overview of the fate of enteric viruses in coastal environments is depicted in Figure 6.3. Domestic sewage (in the form of raw sewage, treated effluent or sewage sludge) may be disposed of directly in the marine environment by coastal outfalls or by dumping from barges. In any case, viruses readily adsorb onto the abundant suspended solids present in the sewage and are discharged solid-associated into the marine environment (Fig. 6.3A). Whereas viruses associated with small-size (<3 μm) particulate material tend to float in the water column (Table 6.5), viruses adsorbed onto large/medium (>6 μm) particles readily settle down in the bottom sediment (Table 6.6). Viruses accumulate in the loose fluffy top layer of the compact bottom sediment (Fig. 6.3B) and are thereby protected from inactivation by natural or artificial processes (Rao et al., 1986; Sobsey et al., 1988). Sediments in coastal seawaters act as reservoirs from which viruses may be subsequently resuspended by several natural or artificial phenomena. Shellfish (Fig. 6.3C), being filter feeders, tend to concentrate viruses and bacteria in their edible tissues, and concentrations of these microorganisms in shellfish may be much higher than in the surrounding water. Shellfish grown in and harvested from waters

receiving urban contaminants (Fig. 6.3D) have been implicated in outbreaks of viral diseases, notably viral hepatitis and gastroenteritis (Halliday et al., 1991; Le Guyader et al., 1996; Christensen et al., 1998; Bosch et al., 2001; Kingsley et al., 2002). Many of these outbreaks were related to water or shellfish meeting legal standards based on bacteriological criteria. This evidence supports the recommendation of monitoring shellfish and their overlying waters for viral contamination including the adoption of guidelines including virus standards.

The possibility nowadays to detect the presence of human enteric viruses in different types of water samples and foodstuff, in particular shellfish samples, should be a valuable tool in the prevention of waterborne and food-borne diseases. Unfortunately, in most outbreaks, virus detection is not attempted until after the outbreak and hence no prophylactic measures can

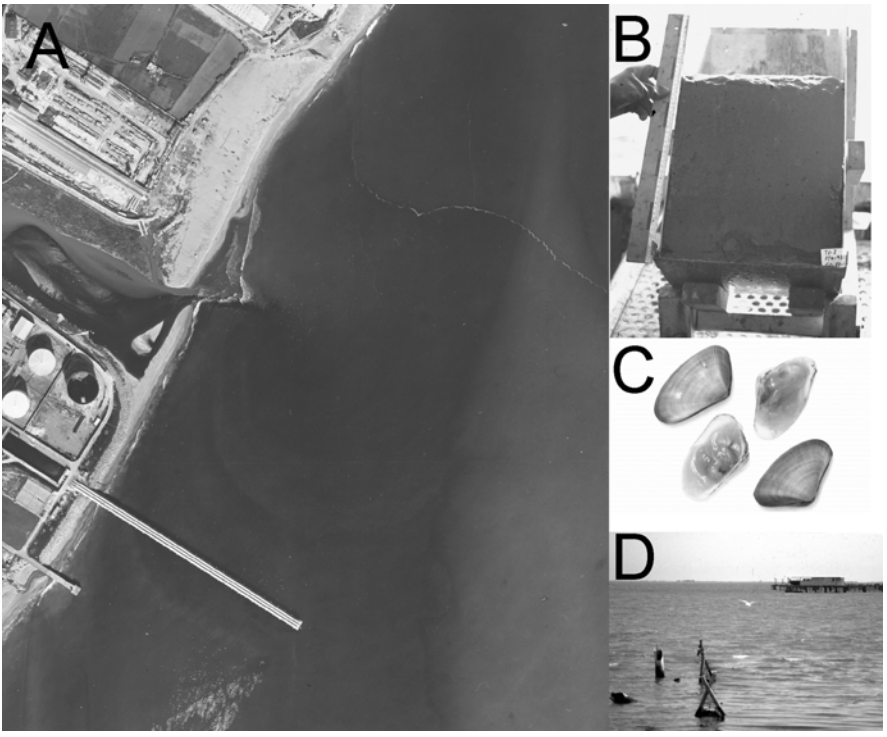

Figure 6.3 Fate of enteric viruses in coastal marine environments. (A) A heavily polluted river with abundant particulate material discharges into the sea. (B) Undisrupted marine sediment with the fluffy top layer where viruses accumulate. (C) Coquina clams and other bivalves readily adsorb pathogenic viruses within their edible tissues. (D) Shellfish grown in areas receiving urban sewage contamination is responsible for outbreaks of gastroenteritis and infectious hepatitis.

Table 6.5 Examples of Human Enteric Virus Isolations from Seawater

Site	*Virus Type*	*Virus Numbers/Liter*	*Reference*
Italy	Enteroviruses	0.4 to 16 $TCID_{50}$	De Flora et al., 1975
USA (Texas)	Enteroviruses	0.01 to 0.44 pfu	Goyal et al., 1979
USA (New York)	Poliovirus Echovirus	0 to 2.1 pfu	Vaughn et al., 1979
France	Enteroviruses Adenoviruses	0.05 to 6.5 MPNCU	Hugues et al., 1980
Spain	Enteroviruses	0.12 to 1.72 MPNCU	Finance et al., 1982
USA (Florida)	Enteroviruses	0.05 to 0.14 pfu	Schaiberger et al., 1982
Israel	Enteroviruses	1 to 6 pfu	Fattal et al., 1983
USA (Texas)	Enteroviruses	0.06 to 0.026 pfu	Rao et al., 1984
Spain	Poliovirus Echovirus	0.12 to 0.15 MPNCU	Lucena et al., 1985
USA (Texas)	Rotaviruses	0.007 to 2.6 pfu	Rao et al., 1986

$TCID_{50}$, tissue culture infectious $dose_{50;}$ MPNCU, Most probable number of cytopathic units; pfu, plaque forming units.

Table 6.6 Examples of Human Enteric Virus Isolations from Marine Sediments

Site	*Virus Type*	*Virus Numbers/Kilogram*	*Reference*
Italy	Enteroviruses Reovirus	0.4 to 40 $TCID_{50}$	De Flora et al., 1975
USA (Florida)	Enteroviruses	0 to 112 pfu	Schaiberger et al., 1982
USA (Texas)	Enterovirus	39 to 398 pfu	Rao et al., 1984
USA (Texas)	Rotavirus	800 to 3800 pfu	Rao et al., 1986
Spain	Enterovirus	5 to 73 pfu	Bosch et al., 1988a
Spain	Enterovirus Rotavirus	130 to 200 pfu 57 to 140 FF	Jofre et al., 1989
Spain	Rotavirus Hepatitis A virus	0 to 560 FF +RNA	Bosch and Pintó, 1992
France	Enterovirus Rotavirus Hepatitis A virus	+RNA +RNA +RNA	Le Guyader et al., 1994

$TCID_{50}$, tissue culture infectious $dose_{50}$; pfu, plaque forming units; FF, fluorescent foci; RNA, detected by molecular hybridization.

be undertaken to decrease the severity of the outbreak. Methods for the detection of viruses in food are discussed elsewhere in this book.

The basic steps in virological analysis of water are sampling, concentration, decontamination/removal of inhibitors, and specific virus detection. Sample concentration is a particularly critical step because the viruses may be present in such low numbers that concentration of the water samples is indispensable to reduce the volume to be assayed to a few milliliters or even microliters. In relatively nonpolluted waters, the virus levels are likely to be so low that optimally hundreds, or even thousands, of liters should be sampled to increase the probability of virus detection.

A good concentration method should fulfill several criteria: it should be technically simple, fast, provide high virus recoveries, be adequate for a wide range of enteric viruses, provide a small volume of concentrate, and be inexpensive. Table 6.7 shows a broad selection of currently available and widely employed procedures; some of them require large equipment. Details on virus concentration procedures have been published elsewhere (American Public Health Association, 1998; Environmental Protection Agency, 1984). All available concentration methodologies have important limitations, and their virus concentration efficiency depends, in part, on the quality of the sampled water. Basically, all available procedures have been evaluated using samples spiked with known viruses. It is known that the recovery efficiency recorded with experimentally contaminated water dramatically decrease when the method is applied in actual field trials. Additionally, none of the existing concentration procedures has been tested with all of the medically important virus groups; normally, a few specific enteric viruses have been employed to conduct the evaluation trials. However, several virus concentration methods have been used successfully to recover naturally occurring enteric viruses in water (Finance et al., 1982; Gerba and Goyal, 1982; Goyal

Table 6.7 Procedures for the Concentration of Viruses from Water Samples

Principle	*References*
Adsorption-elution methods	
Negatively charged filters	Farrah et al., 1976
Positively charged filters	Sobsey and Jones, 1979; Gilgen et al., 1997
Glass powder	Gajardo et al., 1991; Sarrette et al., 1977; Schwartzbrod and Lucena, 1978
Glass fiber	Vilaginès et al., 1997
Precipitation methods	
Organic flocculation	Katzenelson et al., 1976
Ammonium sulfate precipitation	Bosch et al., 1988b; Shields and Farrah, 1986
Polyethylene glycol hydroextraction	Farrah et al., 1978; Lewis and Metcalf, 1988
Ultracentrifugation	Mehnert et al., 1997; Steinman, 1981
Lyophilization	Gajardo et al., 1995; Pintó et al., 2001
Ultrafiltration	Divizia et al., 1989b

and Gerba, 1983; Environmental Protection Agency, 1984; Rao et al., 1986; Lewis and Metcalf, 1988; Henshilwood et al., 1998).

Most of the procedures for concentrating and extracting viruses make use of the properties of the viral proteinaceous macromolecules. Certain protein structures confer on viruses in an aquatic environment the properties of a hydrophilic colloid of an amphoteric nature whose electric charge varies according to the pH and the ionic force of the environment. Viruses can therefore be adsorbed onto and then detach themselves from different substrates that are positively or negatively charged depending on their pH. Methods based on the adsorption of viruses from the sampled water onto a suitable solid surface from which they may subsequently be eluted into a much smaller volume are preferred for use with large-volume samples.

Different types of filters have been evaluated for the recuperation of aquatic viruses, in the form of flat membranes or cartridges. Cartridge-type filters have the advantage to allow filtration of large volumes of moderately turbid water within a relatively short time. Their chemical composition, and porosity vary enormously. A whole range of "negatively" or "positively" charged filters now exist. Their efficiency depends on the type of water being tested and the presence of interfering substances such as detergents, suspended solid matter, or organic matter, which can affect the adsorption of viruses on these filters (Sobsey and Glass, 1984; Sobsey and Hickey, 1985, Gilgen et al., 1997).

The disadvantage of the negatively charged membranes or cartridges (Farrah et al., 1976) is that the water sample must be pretreated prior to concentration. This includes acidification of water and addition of salts to the water sample to facilitate virus adsorption because electronegative filters do not adsorb viruses well under ambient water conditions (Rao and Melnick, 1986). The necessity of this pretreatment step limits the on-location use of this method to a certain extent, although automatic injection systems do exist for treating several hundred liters of water. Virus concentration with electropositive filters may be performed on location at ambient conditions and without any prior amendment of the sample, which make this procedure most suited for in-field studies, provided that the sample pH is lower than 8.5 (Sobsey and Jones, 1979). Glass powder (Sarrette et al., 1977; Schwartzbrod and Lucena, 1978; Gajardo et al., 1991) or glass fiber (Vilaginès et al., 1997) have also been satisfactorily used in different laboratories as adsorbent materials for virus concentration.

Viruses in eluate volumes too large to be conveniently and economically assayed directly for viruses, such as those obtained from processing large volumes of water through cartridge or large disk filters, can be reconcentrated by several methods. Obviously, the recovery of small quantities of viruses from natural waters is dependent not only on the efficacy of primary concentration from the original large volume but also on the reconcentration of the primary eluate to a smaller volume.

Methods such as aluminum hydroxide adsorption-precipitation (American Public Health Association, 1998), polyethylene glycol hydroextraction

(Farrah et al., 1978; Lewis and Metcalf, 1988), organic flocculation (Katzenelson et al., 1976), and ammonium sulfate precipitation (Shields and Farrah, 1986; Bosch et al., 1988b) that are impractical for processing large fluid volumes are suitable for second-step concentration procedures. Alternatively, viruses can be sedimented depending on their molecular weight using ultracentrifugation (Steinman, 1981; Mehnert et al., 1997). Freeze-drying of samples (Gajardo et al., 1995; Pintó et al., 2001) and rehydration in a smaller volume provides a procedure for both virus concentration and removal of PCR inhibitors. Ultrafiltration (Divizia et al., 1989b) can utilize size exclusion rather than adsorption and (or) elution to concentrate viruses can provide consistent recoveries of different viruses under widely varying water conditions.

Because an evaluation of the presence of viruses in sediment provides an additional insight into long-term water-quality conditions, several methods for the detection of viruses have been developed. These methods consist of virus elution from the solid materials followed by concentration of the eluted viruses. Viruses are usually eluted from the sediments by using alkaline buffers (Gerba et al., 1977; Bosch et al., 1988a; Jofre et al., 1989) or chaotropic agents (Wait and Sobsey, 1983; Lewis and Metcalf, 1988; Jofre et al.,1989). Procedures such as organic flocculation (Wait and Sobsey, 1983), ammonium sulfate precipitation (Jofre et al., 1989), polyethylene precipitation (Lewis and Metcalf, 1988), or ultrafiltration (Gerba et al., 1977) are commonly employed to concentrate viruses from the eluate.

1.3. Viruses in Soil

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

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

Table 6.8 Factors Influencing Virus Transport in Soil

Factor	*Effects*
Flow rate	Rate of movement increases with increased flow rate of water.
Hydraulic condition	Rate of movement is greater in saturated than unsaturated soil flow.
Soil texture	Fine-grained soils retain more viruses than coarse-grained soils.
Soil solution	Greater ionic strength means greater adsorption of viruses.
pH	Higher pH leads to greater adhesion to soil.
Virus type	Adsorption varies according to the strain and type of virus.
Humic substances	Organic matter may retard virus adhesion to soil.
Cations	Adsorption increases in the presence of cations.

The most relevant factors controlling virus transport through soil are soil type, water saturation state, pH, conductivity of the percolating water, and soluble organic matter (Table 6.8). The type of soil has a great influence on the level of viral transport. Fine-textured soils tend to absorb viruses more readily than coarsely textured soils. As a general rule, sandy soils are relatively poor adsorbents of enteric viruses, whereas soils with clay content of 30% to 100% are excellent adsorbents (Sobsey et al., 1980). In consequence, viral adsorption increases with increasing clay mineral content (Gerba et al., 1981). The high adsorptive properties of a clay soil will prevent virus transport to another matrix, such as groundwater, whereas coarse soil will not.

Microbial movement in soils is also greatly dependent on the water saturation state. When the soil is saturated, all pores are filled with water, which allows faster virus transport through the soil because virus contact with the soil has been diminished. When the flow is unsaturated, the viruses are in closer contact with the soil, thus promoting virus adsorption to the soil (Santamaría and Toranzos, 2003).

Goyal and Gerba (1979) considered soil pH as the single most important factor influencing viral adsorption, although the combined effect of organic matter and clay content, and cation-exchange capacity, could surpass the sole soil pH effect. At ambient conditions, viruses are usually negatively charged, thus being attracted to and entrapped by positively charged material in soil (Sobsey et al., 1980). In neutral and alkaline soil situations, viruses will not bind to any particulate matter and will be allowed to move freely in soil. There are, however, many exceptions to these general rules.

Virus absorption to soil is also affected by cation concentration. Cations favor virus adsorption to soil by reducing their repulsive forces. Sewage wastes provide an environment that enhances virus retention to soil, while this retention would be low in distilled water. As a matter of fact, distilled water may actually lead to the elution of viruses from soils, favoring virus

mobilization and transport through soil. On the other hand, soluble organic matter will compete with the virus for soil adsorption sites. Likewise, humic and fulvic acids will also compete with the virus and will reduce the level of adsorption of viruses to the soil (Sobsey and Hickey, 1985).

2.0. VIRUS PERSISTENCE IN THE ENVIRONMENT

Persistence is the term of choice to describe the capacity of a given virus to retain its infectivity in a given scenario. However, some authors unfamiliar with environmental virology claim that this term is confusing because it also describes the ability of certain viruses to produce infections in which, contrary to what applies in acute infections, a degree of equilibrium is established between the virus and the host (i.e., a cell or a whole animal). Other authors avoid the use of the term survival to describe the natural persistence of virus infectivity, based on the ambiguity of the "live" condition of viruses. Keeping in mind that a virus will be able to maintain its infectious status provided that all the virion components remain unaltered, the term *stability* may also be properly employed in this context.

One critical question in environmental virology is whether or not viruses can persist long enough, and in high enough concentrations in the environment, to cause disease in individuals who are in contact with polluted recreational water, soil, or fomites, or who consume contaminated water or seafood. Because viruses outside their hosts are inert particles, their chances of transmission from host to host are greatly dependent on the degree of their robustness, which allows them to remain infectious during the various conditions they may encounter in the environment.

Numerous physical, chemical, and biological factors influence virus persistence in the environment (Table 6.9). Some of the primary factors affecting the survival of viruses in liquid environmental matrices or media are temperature, ionic strength, chemical constituents, microbial antagonism, the sorption status of the virus, and the type of virus. Considerable differences have been observed in the survival of viruses in different types of environmental samples. Different behaviors and inactivation rates have been observed not only among viruses of different families and genera, but also among viruses of the same family, genus, and even among similar types or strains of virus (Block, 1983).

Among the chemical constituents of liquid or semisolid (feces, human night soil, biosolids, animal manures, etc.) environmental matrices, the amount and type of organic matter and specific antiviral chemicals (such as ammonia at elevated pH levels) play a role in virus stability. Of the physical factors influencing virus persistence in liquid media, temperature, sunlight, and virus association with solids are among the most important. Soil moisture, temperature, sunlight, and other soil characteristics may influence the persistence of viruses in soil. On inanimate surfaces, the most important factors that affect virus stability are the type of virus and surface, relative

Table 6.9 Factors Affecting Virus Persistence in the Environment

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

humidity, moisture content, temperature, composition of the suspending medium, light exposure, and presence of antiviral chemical or biological agents. Most of these factors are also relevant for the ability of viruses to persist in aerosolized droplets, together with the moisture content and the size of the aerosol particles, and the air quality.

Some enteric virus infections follow a seasonal pattern, whereas others fail to do so. In regions with temperate climates, infections due to enteroviruses generally reach a peak in summer and early fall (Moore, 1982). On the contrary, rotavirus, norovirus, and astrovirus infections occur mainly during the cooler months (McNulty, 1978; Mounts et al., 2000; Guix et al., 2002), although seasonal and nonseasonal distributions of rotavirus in sewage have been described (Hejkal et al., 1984; Bosch et al., 1988c). On the other hand, cases of hepatitis A do not show a clear seasonal pattern (Lemon, 1985), whereas enteric adenovirus infections are reported to peak in midsummer (Wadell et al., 1989). These data suggest that temperature, and probably relative humidity, may be meaningful in the seasonal distribution of outbreaks of certain human enteric viruses (Enright, 1954), due to the influence of these factors on virus persistence.

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

studies involving the persistence of enteric viruses in the absence of any deliberately applied inactivation process are reviewed. Neither work on virus disinfection nor studies conducted with potential indicators, such as bacteriophages, are considered because they will be discussed in other chapters.

2.1. Methods to Study Environmental Virus Persistence

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

Molecular detection approaches such as PCR or RT-PCR are normally employed for fastidious virus analysis. However, they are unable to differentiate between infectious and noninfectious particles (Kopecka et al., 1993; American Public Health Association, 1998) and are, therefore, unsuitable for virus persistence studies, even when quantitative real-time procedures are employed. Although reports on the presence of norovirus sequences in bottled mineral water raised a lot of concern (Beuret et al., 2000, 2002), many authors have shown the lack of correlation between virus persistence and molecular detection of virus genomes. It now seems obvious that infectious particles are degraded more rapidly than virus genomes.

Most enteric viruses of public health concern consist of RNA genomes. In studies employing RT-PCR, it has been shown that poliovirus genomic RNA is not stable in nonsterilized water (Tsai et al., 1995). Although free DNA is fairly stable, it is unlikely that a free single-stranded RNA genome of noroviruses, astrovirus, poliovirus, or hepatitis A virus would remain stable without its protein coat in the environment. This presumption is less clear for the double-stranded RNA genome of rotaviruses. Nevertheless, it has been shown that altered nucleocapsids of noninfectious virions may still encapsidate a RT-PCR detectable single-stranded RNA (Gassilloud et al., 2003).

Amplification of a piece of the virus genome is not indicative of the presence of the infectious agent. It can be assumed that even when different target sequences from unrelated parts of the genome are detected by molecular amplification, there is still no indication of the presence of unaltered capsid with functional surface residues involved in receptor recognition and cell attachment.

From a strictly theoretical point of view, the use of an antigen-capture PCR assay, involving virus binding with a conformationally dependent monoclonal antibody and amplification of different unrelated genomic targets, could provide a fair estimation on the stability of a nonculturable virus. However, this approach requires a lot of experimentation before it can be

considered adequate to be applied in virus persistence studies. In the meantime, infectious surrogates are usually employed to generate data on unculturable virus survival; for example, feline calicivirus has been used to mimic norovirus behavior (Thurston-Enriquez et al., 2003a, 2003b). Another promising approach to increase the likelihood of detecting intact and potentially infectious viruses in cell cultures is to pretreat the virions with proteolytic enzymes and nucleases prior to nucleic acid extraction, amplification, and detection, thereby eliminating the detection of free nucleic acids or nucleic acids associated with damaged, inactivated virions (Nuanualsuwan and Cliver, 2002).

Some health significant enteric viruses, such as rotavirus, astrovirus, and enteric adenovirus, replicate poorly in cell cultures; yet their persistence may be evaluated by integrated cell culture RT-PCR assays (Pintó et al., 1995; Reynolds et al., 1996; Abad et al., 1997; Reynolds et al., 2001). For this purpose, cells supporting the propagation of a wide variety of enteric viruses, such as CaCo-2 (colonic carcinoma) or PLC/PRF/5 cells (human liver hepatoma), are used for an *in vivo* amplification step prior to molecular amplification (Grabow et al., 1993; Pintó et al., 1994). It should be recognized, however, that most of the studies on virus persistence in the environment were performed under laboratory conditions and that data obtained from these studies may not truly represent their behavior under actual field conditions.

2.2. Virus Persistence in Environmental Waters

The survival of viruses in environmental waters has been extensively reviewed (Bitton, 1980; Kapucinski and Mitchell, 1980; Block, 1983; Bosch, 1995). As previously mentioned, the most relevant factors affecting virus survival in the water environment are temperature (Akin et al., 1971; Raphael et al., 1985; Bosch et al., 1993), virus association with solids (Gerba and Schaiberger, 1975; La Belle et al., 1980; Rao et al., 1984; Sobsey et al., 1988), exposure to UV (Bitton et al., 1979; Bitton, 1980), and the presence of microbial flora (Gunderson et al., 1968; Fujioka et al., 1980; Toranzo et al., 1983; Ward et al., 1986; Gironés et al., 1989, 1990).

The effect of temperature on viral persistence in water may be due to several mechanisms including protein denaturation, RNA damage, and influence on microbial or enzymatic activity (Dimmock, 1967; Melnick and Gerba, 1980; Deng and Cliver, 1995). Early studies pointed to damage to virion proteins as the primary target for viral inactivation at high temperatures, although damage to both protein and RNA occurs at all temperatures (Dimmock, 1967). Even though all viruses persist better at lower temperatures than at higher temperatures, some viral strains, such as hepatitis A virus and parvovirus, do exhibit higher thermal resistance than other viruses.

As mentioned earlier in this chapter, virus adsorption to particulate material increases the persistence of enteric viruses in the water environment (Gerba and Schaiberger, 1975; La Belle et al., 1980; Rao et al., 1984; Sobsey et al., 1988), although differences have been observed among study locations

(La Belle et al., 1980). The increased virus survival in the presence of sediment has important implications in the marine environment, because fecal contamination of coastal areas results in contamination of shellfish harvesting areas, accumulation of solid-associated viruses in sediments with sediments acting as virus reservoirs, and finally accumulation of viruses in shellfish. Additionally, virus uptake by molluskan bivalves is enhanced by the presence of particulate material (Landry et al., 1983).

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

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

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

2.3. Virus Persistence in Soil

As has been mentioned earlier, soil pollution with human wastes may greatly contribute to groundwater contamination. Because of the increasing empha-

sis placed on land application as a means of organic waste disposal, it appears relevant to evaluate the persistence of human pathogens in soil.

Viruses in moisture-saturated soils may remain infectious for long periods of time, even at ambient temperatures of 20°C, in which the soil would be microbially active. If soil moisture drops under 10%, dramatic losses in virus infectivity are observed regardless of soil temperature or the medium in which viruses are applied (Yeager and O'Brien, 1979). For example, enteric viruses survive for 15–25 days in an air-dried soil as compared with 60–90 days in soils with 10% moisture content. One of the pathways for virus removal from warm soils is through evaporation, which would account for the loss of viral pathogens from dry soils. The rate of evaporation is directly related to temperature and relative humidity. Under constant moisture of 10% or greater, the main factors controlling the inactivation of viruses appear to be not only soil temperature but also soil texture. The survival of viruses is enhanced by a combination of low soil temperature and sufficient moisture (Bitton, 1980). As temperature increases, the virus inactivation rates also increase significantly (Yeager and O'Brien, 1979; Straub et al., 1992). At 4°C and with constant moisture, viruses are able to persist for 180 days, whereas at 37°C no viruses persist after 12 days.

Certain soil characteristics also influence virus survival. For example, virus persistence has been reported to decrease as a function of increasing soil pH and resin-extractable phosphorus (Hurst et al., 1980). Increase in exchangeable aluminum, on the other hand, increased virus survival. The relative levels of clay and humic acids may also enhance virus survival (Bitton and Gerba, 1984). Viruses survive better in an adsorbed state than in suspension. Virus adsorption to clay materials through electrostatic interactions is speculated to protect viral genome against nucleases or other antagonistic factors in soil (Bitton and Gerba, 1984). Additionally, clay contributes to virus survival by retaining minimum amounts of water, even in dry soils. This water provides the moisture required for virion stability. On the other hand, poorly absorbent sandy soils can increase their viral retention in the presence of divalent cations (Mg^{2+}, Ca^{2+}) but not monovalent (Na^{+}) or trivalent (Fe^{3+}) cations (Lefler and Kott, 1974).

Clay loam soils generally afford more protection to viruses than sandy soils. However, in rapidly drying soils, virus persistence may decrease more deeply in clay soils than in sandy soils, due to the water-holding capacity of soil. Clay soils can hold more water than sandy soils, but when water is evaporating from both soils, the clay soils, because of their mineral content, will retain the remaining water more tightly than sandy soils at the same moisture content, making them less apt for biological activity (Straub et al., 1992).

The presence of indigenous microorganisms is deleterious to virus survival, although this effect is not observed at low temperature; at 1°C poliovirus remains stable through 70 days (Hurst et al., 1980). Indigenous soil aerobic microorganisms significantly reduce virus persistence, while indigenous anaerobes do not (Hurst, 1987). In a study involving a variety of soils and poliovirus, echovirus and HAV suspended in groundwater, second-

ary sewage effluent or primary sewage treatment effluent, HAV was usually more persistent than poliovirus and echovirus; the 99% reduction times for HAV were normally greater than 12 weeks (Sobsey et al., 1989). This indicates that HAV is an extremely stable agent, capable of persisting for more than 3 months in soil, and hence it poses a health threat.

The ultraviolet component of sunlight is destructive to viruses (Bitton, 1980). The UV has been shown to inactivate viruses at the surface of the soil but as the viruses move deeper in the soil column, it plays a minor role in inactivating viruses. Disposable diapers may contribute to soil contamination with human pathogens. A field survey of virus inactivation in diapers buried in landfills for at least 2 years showed complete inactivation (Huber et al., 1994). In laboratory conditions, HAV and poliovirus experimentally seeded in disposable diapers showed 2.5 and 4 $\log_{10}$ reduction, respectively, after 80 days at 25°C, in aerobic conditions (Gray et al., 1993).

Quantitative interpretations (Carrington et al., 1998a, 1998b) of existing data on poliovirus and cytopathic enterovirus decay rates in sludge-amended soil (Tierney et al., 1977; Hurst et al., 1978) indicated that, at the prevailing summer temperatures (19–34°C), the decimal reduction rates were between 2.7 and 3.7 days, whereas at the winter temperatures (13–26°C), it was 24 days. Carrington et al. (1998a) analyzed data reported by Straub et al. (1993) and found that decimal reduction time for poliovirus at winter temperature of 15°C and moisture levels of 15–25% was 92 days as compared with 1.2 days at summer temperatures of 27–33°C at moisture levels of 3–40%.

Most studies on virus persistence in soil have been performed in North American soil types and autochthonous climatic conditions. It has been pointed out that in other parts of the world, where mean soil temperature seldom exceeds 15°C at 10 cm depth in summer and about 5°C in winter, viral decay rates would be slow or with decimal reduction times from 24 days to more than 100 days (Carrington et al., 1998b; Rzezutka and Cook, 2004). However, the same authors suggest that cultivation of soil after sludge application would encourage virus decay by enhancing evaporation.

2.4. Virus Persistence on Fomites

Outbreaks of acute gastroenteritis and hepatitis are a matter of concern in institutions such as daycare centers, hospitals, nurseries, schools, and military quarters. Many of these outbreaks have been suspected to be caused by vehicular transmission of agents through contaminated environmental surfaces (Ryder et al., 1977; Halvorsrud and Orstavick, 1980; Rocchi et al., 1981; Sattar et al., 1986; Butz et al., 1993; Green et al., 1998). As has been mentioned earlier, stools from patients with diarrhea or hepatitis contain a very high number of the causative virus, and a single vomiting episode of an individual suffering from norovirus gastroenteritis may expel 3×10^7 virus particles, all of which are able to contaminate fomites (Cheesbrough et al., 1997; Green et al., 1998, 1994).

It has been demonstrated that human enteric viruses are able to survive on several types of materials commonly found in institutions and domestic

environments long enough to represent a source for secondary transmission of disease (Hendley et al., 1973; Sattar et al., 1986, 1987; Ansari et al., 1988; Mbithi et al., 1991; Abad et al., 1994, 2001). The stability of health-significant human enteric viruses has been investigated on various nonporous (aluminum, china, glazed tile, plastic, latex, stainless steel, and polystyrene) and porous (cloth, different types of papers and cotton cloth) surfaces (Sattar et al., 1986; Abad et al., 1994, 2001). As a general conclusion, when dried on environmental fomites, hepatitis A virus and rotavirus are more resistant to inactivation than enteric adenovirus, astrovirus, and poliovirus.

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

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

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

As mentioned previously, there is a considerable shedding of the SARS coronavirus in stools, where it remains stable at room temperature for several days (Tsang, 2003). Although epidemiological evidence suggests that the major mode of transmission for SARS coronavirus is by close personal contact with an infected individual, contact with environmental surfaces contaminated with respiratory secretions or other body fluids may also play a role in transmission (Tsang, 2003). In addition, SARS coronavirus has been detected in a variety of environmental surfaces, such as the toilet and floor in the aparment of an infected individual and the walls and rooftop of a building with multiple cases (Tsang, 2003).

Hands are frequently in contact with environmental surfaces, and the potential for transfer of virus between surfaces and hands has been studied

(Hendley et al., 1973; Ansari et al., 1988; Mbithi et al., 1992). It was ascertained in these studies that rotavirus and hepatitis A virus could retain infectivity for several hours on skin and could be transferred in an infectious state from fingertips to other surfaces and vice versa. Enteric virus transfer between hands was apparently influenced by moisture. Moisture would mediate suspension of virus particles and facilitate their movement between touching surfaces; drying would reduce this effect. Laboratory studies have shown that viruses persist better in the environment at high relative humidity and at low temperatures (Moe and Shirley, 1982; Sattar et al., 1988; Sobsey et al., 1988; Abad et al., 1994). However, data on the effect of relative humidity on enteric virus survival is contradictory. These reported differences, particularly affecting rotavirus persistence, are difficult to explain but may be due to differences in the methodologies between these studies.

Temperature substantially affects the survival of feline calicivirus, an infectious surrogate for human norovirus, which is able to persist for long periods of time dried onto glass coverslips with log reductions of 4.75 after 2 months and 3 weeks, at 4°C and room temperature, respectively (Doultree et al., 1999). The authors suggested that the effect of temperature on feline calicivirus stability may reflect the greater prevalence of norovirus infections in cooler seasons (Lopman et al., 2003).

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

2.5. Virus Persistence in Aerosols

Aerosols are an important means of virus transmission in humans. Various authors have reported the isolation of enteric viruses from aerosols produced by sludge-treatment plants (Fannin et al., 1985; Fattal et al., 1987; Pfirrmann and Bossche, 1994; Alvarez et al., 1995). The presence of microorganisms in aerosols generated from wastewater-treatment processes or in treated wastewater for agricultural irrigation is a potential danger to human health (Teltsch et al., 1980; Alvarez et al., 1995). In hospitals, aerosolization of vomit was reported to be of major importance in the transmission of norovirus infection during outbreaks, while cleaning vomit or feces from patients did not significantly increase the risk of developing gastroenteritis (Chadwick and McCann, 1994). Members of the *Caliciviridae* family have been reported to be fairly stable in aerosols (Donaldson and Ferris, 1976). The most important factors affecting the stability of viruses in the aerosol state are temperature, pH, relative humidity, moisture content, size of the aerosol particle, composition of the suspending medium, sunlight exposure, air quality, and virus type.

The basis of virus inactivation in aerosols is poorly understood, although mechanisms for bacteriophage inactivation in aerosols have been proposed

(Trouwborst et al., 1974). At high relative humidity, surface alteration of the virion has been reported, whereas at low relative humidity virus inactivation appears to be mediated by the removal of structural water molecules. Relative humidity seems to confer a protective effect on aerosolized nonenveloped virus particles. Thus, poliovirus was more stable in aerosol at 22°C at high relative humidity than at low relative humidity (Hemes et al., 1960; Harper, 1961). Picornavirus infectious RNA may be detected at all humidity levels, suggesting that virus inactivation is caused by virion capsid damage (Akers and Hatch, 1968).

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

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

2.6. Virus Persistence in Food

Outbreaks of viral infection attributed to the consumption of contaminated soft fruit, salad vegetables, and other foods are increasingly reported (Mead et al., 1999; Lopman et al., 2003). A recent example is an outbreak of hepatitis A virus in western Pennsylvania in late 2003, which affected more than 600 people and resulted in three fatalities (MMWR, 2003). The incident involved green onions imported from Mexico and added to the restaurant's homemade salsa. Those green onions were stored in a single container for up to 5 days in the ice used for shipping them. Some of the uncooked green onions were used in the restaurant's mild salsa that was prepared in large batches and stored for up to 3 days. If the shipment ice was contaminated, prolonged exposure combined with the relatively long storage of salsa may account for why so many patrons became infected. Green onions, which are multilayered and can retain soil particles that could harbor fecal contaminants, were probably contaminated during harvesting and packing. Alternatively, HAV-contaminated water used for irrigation, processing, and storage

Table 6.10 Examples of Virus Persistence in Food

Food	*Virus*	*Temperature*	*Storage Time*	*Log_{10} Titer Reduction*	*Reference*
Lettuce	HAV	4°C	7 days	2.03	Croci et al., 2002
Carrot	HAV	4°C	7 days	≥2.44	
Lettuce	HAV	RT	6 days	≤1.00	Bidawid et al., 2001
		4°C	6 days	≤0.50	
		RT	12 days	4.00	
		4°C	12 days	≤0.50	
Cabbage	Polio	8–17°C	5 days	6.15	Ward et al., 1982
		13–22°C	2 days	5.55	
Grass[a]	Polio	4–16°C	40 hr	2.40	Badawy et al., 1990
	Rotavirus	4–16°C	40 hr	≥4.87	
	Polio	22–41°C	40 hr	≥4.39	
	Rotavirus	22–41°C	40 hr	2.99	
Creme sandwich	HAV	21°C	7 days	2.05	Sobsey et al., 1988

RT, room temperature.
[a] Bermuda hybrid grass and rye grass.

may have been the source of contamination. The high environmental persistence of HAV makes any of these scenarios possible.

Data on the potential of enteric viruses to persist between the preparation of food and its consumption are required to ascertain the risk of virus transmission through food. This information is also important for the development of treatments applied to food in order to inactivate contaminant viruses. Disinfection practices for food are reviewed in another chapter.

Examples of studies on virus persistence in food are depicted in Table 6.10. Studies have shown that viruses remain infectious for several days or weeks on vegetable crops irrigated with contaminated sewage effluent or sludge (Tierney et al., 1977; Ward and Irving, 1987). Several enteroviruses have been reported to survive during commercial and household storage for periods of up to 5 weeks on vegetables irrigated with contaminated effluent (Larkin et al., 1976; Ward and Irving, 1987).

The factors that affect virus survival in the environment, especially on fomites, are also relevant for the fate of viruses in food products. Among them, temperature has a great influence on virus stability in food as in any other suspending matrix; the higher the temperature, the more pronounced the virus decay. Natural or added constituents of food may influence the rate of virus inactivation by temperature (Cliver and Riemann, 1999). For instance, salt used in pickling sausage batter has been shown to protect viruses from thermal inactivation (Grausgruber, 1963), whereas acidity often enhances the virucidal effect of temperature (Cliver et al., 1970; Salo and Cliver, 1976). Additionally, viruses appear to resist thermal inactivation during cooking when fat levels are high (Filippi and Banwart, 1974).

Furthermore, some ingredients may have a direct virucidal effect, as has been elucidated for free, unsaturated fatty acids with enveloped viruses (Kohn et al., 1980). Naturally occurring substances in fruit juices have been reported to bear a reversible inactivating effect on enteroviruses, attributed to plant polyphenols such as tannins (Konowalchuk and Speirs, 1976, 1978; Cliver and Kostenbader, 1979).

Although the presence of fecal material and high relative humidity strongly enhances virus persistence (Konowalchuk and Speirs, 1975), the effect of modified atmosphere packaging does not appear to be significant on virus persistence (Bidawid et al., 2001). As is the case with fomites, a rapid and marked decline in virus titer on crops/vegetables is attributed to drying/desiccation (Larkin et al., 1976; Tierney et al., 1977; Ward and Irving, 1987) combined with the action of sunlight and temperature (Kott and Fishelson, 1974). Direct sunlight irradiation (particularly its UV component) by itself is able to induce a pronounced reduction in virus numbers in food (Badawy et al., 1990).

3.0. CONCLUSIONS

Further work is required to develop robust and reliable quantitative methods to recover and detect health significant viruses in environmental and food samples. These procedures should also be adequate for newly recognized emerging pathogens of concern, as well as for non-human viruses capable of zoonotically infecting humans and having greater potential to cause human infection and illness. Simple standardized diagnostic procedures for selected pathogens are needed to establish specific virological guidelines in selected food products, notably shellfish or food imports from regions with endemic infections.

Molecular characterization of agents responsible for waterborne and food-borne outbreaks will provide relevant information on the prevalence of infections among the population, which may be important in the development and/or efficacy of vaccines. The long pursued objective of the eradication of poliomyelitis will require comprehensive surveys on the occurrence of wild-type and vaccinal-type poliovirus in environmental samples that may represent potential reservoirs and vehicles of transmission.

Another important issue in environmental studies is microbial source tracking, which is imperative for the maintenance of microbiological quality and safety of water systems used for drinking, recreation, and in seafood harvesting, because contamination of these systems can represent high risks to human health and significant economic losses due to closure of beaches and shellfish harvesting areas. As mentioned earlier and discussed elsewhere in this book, bacteriophages and other microorganisms of fecal flora have been proposed as models of virus behavior. However, from the strictly structural point of view, there is no better surrogate of an actual virus pathogen to track their behavior in the environment than a noninfectious virus-like particle

(VLP) of the same virus. Recombinant VLPs of health-significant viruses as norovirus and rotavirus have been employed to investigate the influence of electrostatic interactions in the filtration of norovirus in quartz sand and rotavirus behavior under disinfection conditions, respectively (Redman et al., 1997; Caballero et al., 2004). As model systems, recombinant tracers are perfectly adequate for field studies of microbial tracking, as they may be produced in extremely high numbers (several milligram amounts). Additionally, their noninfectious nature, due to the lack of a nucleic acid, makes them suitable for use in scenarios where the use of actual pathogenic viruses is not prudent; for example, drinking-water treatment plants, shellfish growing waters, or selected food products.

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