CHAPTER 6

Survival and Transport of Enteric Viruses in the Environment

Albert Bosch, Rosa M. Pintó, and F. Xavier Abad

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
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 pre-
vented bacterial infections, but 30,000 cases of hepatitis occurred among the 
population. As a consequence of this outbreak, studies in water and envi-
ronmental virology began with efforts to detect poliovirus in water around 
50 years ago. Since that time, other enteric viruses responsible for gastroen-
teritis and hepatitis have replaced enteroviruses as the main target for detec-
tion 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 individ-
uals; 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

| 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                                                                |

Table 6.1 Human Enteric Viruses with Potential Environmental Transmission
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](image-url)  
*Figure 6.1* Routes of enteric virus transmission. Thick and thin arrows depict the main and minor routes of virus transmission, respectively.
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  | Gastroenteritis | Foy et al., 1968; D’Angelo et al., 1979                                  |
|                         |             | Hepatitis A     | Birch and Gust, 1989                                                      |
| Recreational freshwater | Coxsackie   | Gastroenteritis | 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                                                        |

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

### 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     | 813          | NoV               | Morse et al., 1986         |
|          |                 | Oysters   | 204          |                   |                            |
| 1988     | China           | Clams     | 292, 301     | HAV               | Halliday et al., 1991      |
| 1999     | Spain           | Clams     | 183          | HAV               | Bosch et al., 2001         |
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 matter 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

---

**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           | 4<sup>a</sup> | Divizia et al., 1989a |
| Undetermined rivers (Germany) | Enteroviruses | 0.5 to 56 | Walter et al., 1989 |
| Saint-Lawrence (Canada) | Culturable enteric viruses<sup>b</sup> | 0.1 to 29 | Payment et al., 2000 |

MPNCU, most probable number of cytopathic units of virus.

<sup>a</sup> Molecular detection of viral RNA.

<sup>b</sup> Unidentified virus grown in MA104 cells and reacting with human immunoglobulin.
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<sub>50</sub> | De Flora et al., 1975  |
| USA (Texas)     | Enteroviruses    | 0.01 to 0.44 pfu    | Goyal et al., 1979      |
| USA (New York)  | Poliovirus       | 0 to 2.1 pfu        | Vaughn et al., 1979     |
| France          | Enteroviruses    | 0.05 to 6.5 MPNCU    | Hugues et al., 1980     |
| France          | Echovirus        |                     |                         |
| 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       | 0.12 to 0.15 MPNCU   | Lucena et al., 1985     |
| Spain           | Echovirus        |                      |                         |
| USA (Texas)     | Rotaviruses      | 0.007 to 2.6 pfu    | Rao et al., 1986        |

TCID<sub>50</sub>, tissue culture infectious dose<sub>50</sub>; 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    | 0.4 to 40 TCID<sub>50</sub> | 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      | 130 to 200 pfu          | Jofre et al., 1989      |
| Spain           | Rotavirus        | 57 to 140 FF            |                         |
| Spain           | Rotavirus        | 0 to 560 FF + RNA       | Bosch and Pintó, 1992   |
| France          | Enterovirus      | + RNA                   | Le Guyader et al., 1994 |
| France          | Rotavirus        | + RNA                   |                         |
| France          | Hepatitis A virus| + RNA                   |                         |

TCID<sub>50</sub>, tissue culture infectious dose<sub>50</sub>; 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, concentra-
tion, 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 re-concentrated 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 re-concentration 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.
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

---

**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.                         |
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
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

| Factor                  | Effect                                                                 |
|------------------------|------------------------------------------------------------------------|
| Physical               | Inactivation is directly proportional to temperature                   |
| Heat                   | 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               | Worst stability at extreme pH values                                   |
| pH                     | Increased salt concentrations are virucidal                            |
| Salinity               | Virucidal                                                              |
| Ammonia                | Some (e.g., Pt, Pd, Rh) are virucidal                                  |
| Inorganic ions         | Dissolved, colloidal, and solid organic matter protect from inactivation|
| Organic matter         | Proteases and nucleases contribute to inactivation                     |
| Enzymes                |                                                                         |
| 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             |
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 sediments 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 nuclease 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 log10 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 $3 \times 10^7$ 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 apartment 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
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).

| 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       | ≥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.
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.

4.0. REFERENCES

Abad, F. X., Pinto, R. M., and Bosch, A., 1994, Survival of enteric viruses on environmental fomites. Appl. Environ. Microbiol. 60:3704–3710.
Abad, F. X., Pintó, R. M., Villena, C., Gajardo, R., and Bosch, A., 1997, Astrovirus survival in drinking water. Appl. Environ. Microbiol. 63:3119–3122.
Abad, F.X., Villena, C., Guix, S., Caballero, S., Pinto, R. M., and Bosch, A., 2001, Potential role of fomites in the vehicular transmission of human astroviruses. Appl. Environ. Microbiol. 67:3904–3907.
Akers, T. G., and Hatch, M. T., 1968, Survival of a picornavirus and its infectious ribonucleic acid after aerosolization. Appl. Microbiol. 16:1811–1813.
Akin, E. W., Benton, W. H., and Hill, J. W. Jr., 1971, Enteric viruses in ground and surface waters: a review of their occurrence and survival, in: Virus and Water Quality: Occurrence and Control (V. Griffin and J. Snoeyink, eds.), University of Illinois Press, Urbana, IL, pp. 59–74.
Alvarez, A., Buttner, M. P., and Stetzenbach, L., 1995, PCR for bioaerosol monitoring: sensitivity and environmental interference. Appl. Environ. Microbiol. 61:3639–3644.
American Public Health Association, 1998, Standard Methods for the Examination of Water and Wastewater, 20th ed., American Public Health Association, American Water Works Association, Water Pollution Control Federation, Washington, DC.
Amvrosieva, T. V., Titov, L. P., Mulders, M., Hovi, T., Dyakonova, O. V., Votyakov, V. I., Kvaicheva, Z. B., Eremin, V. F., Sharko, R. M., Orlova, S. V., Kazinets, O. N., and Bogush, Z. F., 2001, Viral water contamination as the cause of aseptic meningitis outbreak in Belarus. Cent. Eur. J. Publ. Health 9:154–157.
Andersson, Y., and Stenström, G., 1987, Waterborne outbreaks in Sweden: causes and etiology. Water Sci. Technol. 19:575–580.
Ansari, S. A., Sattar, S. A., Springthorpe, V. S., Wells, G. A., and Tostowaryk, W., 1988, Rotavirus survival on human hands and transfer of infectious virus to animate and nonporous inanimate surfaces. J. Clin. Microbiol. 26:1513–1518.
Appleton, H., and Pereira, M. S., 1977, A possible virus aetiology in outbreaks of food poisoning from cockles. Lancet 1:780–781.
Badawy, A. S., Rose, J. B., and Gerba, C. P., 1990, Comparative survival of enteric viruses and coliphage on sewage irrigated grass. J. Environ. Sci. Health 25:937–952.
Banks, M., Bendall, R., Grierson, S., Heath, G., Mitchell, J., and Dalton, H., 2004, Human and porcine hepatitis E virus strains, United Kingdom. Emerg. Infect. Dis. 10:953–955.

Benbough, J. E., 1969, The effect of relative humidity on the survival of airborne Semliki forest virus. J. Gen. Virol. 4:473–437.

Beuret, C., Kohler, D., and Lüthi, T. M., 2000, Norwalk-like virus sequences detected by reverse transcription-polymerase chain reaction in mineral waters imported into or bottled in Switzerland. J. Food Prot. 63:1576–1582.

Beuret, C., Kohler, D., Baumgartner, A., and Lüthi, T. M., 2002, Norwalk-like virus sequences in mineral waters: one-year monitoring of three brands. Appl. Environ. Microbiol. 68:1925–1931.

Bidawid, S., Farber, J. M., and Sattar, S. A., 2001, Survival of hepatitis A virus on modified atmosphere-packaged (MAP) lettuce. Food Microbiol. 18:95–102.

Birch, C., and Gust, I., 1989, Sewage pollution of marine waters: the risks of viral infection. Med. J. Aust. 151:609–610.

Bitton, G., 1980, Introduction to Environmental Virology, John Wiley & Sons, New York.

Bitton, G., and Gerba, C. P., 1984, Groundwater pollution microbiology: the emerging issues, in Groundwater Pollution Microbiology (G. Bitton and C.P. Gerba, eds.), John Wiley & Sons, New York. pp. 1–8.

Bitton, G, Fraxedas, R., and Gifford, G. E., 1979, Effect of solar radiation on poliovirus: preliminary experiments. Water Res. 13:225–228.

Blacklow, N. R., and Cukor, G., 1982, Norwalk virus: a major cause of epidemic gastroenteritis. Am. J. Public. Health. 72:1321–1323.

Block, J. C., 1983, Viruses in environmental waters, in: Viral Pollution of the Environment (G. Berg, ed.), CRC Press, Inc., Boca Raton, FL, pp. 117–145.

Bosch, A., 1995, The survival of enteric viruses in the water environment. Microbiologia SEM 11:393–396.

Bosch, A., and Pintó, R. M., 1992, Human enteric viruses in the environment, in: Environmental Protection, Vol. 3 (A.Z. Keller and H.C. Wilson, eds.), University of Bradford Press, Bradford, UK, pp 63–71.

Bosch, A., Lucena, F., Girones, R., and Jofre, J., 1986, Survey of viral pollution in Besos River. J. Water Pollut. Control Fed. 58:87–91.

Bosch, A., Lucena, F., Girones, R., and Jofre, J., 1988a, Occurrence of enterovirus on marine sediment along the coast of Barcelona (Spain). Can. J. Microbiol. 34:921–924.

Bosch, A., Pintó, R. M., Blanch, A. R., and Jofre, J., 1988b, Detection of human rotavirus in sewage through two concentration procedures. Water Res. 22:343–348.

Bosch, A., Pintó, R. M., and Jofre, J., 1988c, Non-seasonal distribution of rotavirus in Barcelona raw sewage. Zbl. Bakt. Hyg. B 186:273–277.

Bosch, A., Lucena, F., Diez, J. M., Gajardo, R., Blasi, M., and Jofre, J., 1991, Waterborne viruses associated with a hepatitis outbreak. J. Am. Water Works Assoc. 83:80–83.

Bosch, A., Gray, M., Diez, J. M., Gajardo, R., Abad, F. X., Pintó, R. M., and Sobsey, M. D., 1993, The survival of human enteric viruses in seawater. MAP Tech. Rep. Ser. 76:1–7.

Bosch, A., Sánchez, G., Le Guyader, F., Vanacllocha, H., Haugarreau, L., and Pintó, R. M., 2001, Human enteric viruses in coquina clams associated with a large hepatitis A outbreak. Water Sci. Tech. 43:61–66.
Bryan, J. A., Lehmann, J. D., Setiady, T. F., and Hatch, M. H., 1974, An outbreak of hepatitis A associated with recreational lake water. *Am. J. Epidemiol.* 99:145–154.

Butz, A. M., Fosarelli, P., Dick, J., Cusack, T., and Yolken, R., 1993, Prevalence of rotavirus on high-risk fomites in day-care facilities. *Pediatrics* 92:202–205.

Caballero, S., Abad, F. X., Loisy, F., Le Guyader, F. S., Cohen, J., Pintó, R. M., and Bosch, A., 2004, Rotavirus virus-like particles as surrogates in environmental persistence and inactivation studies. *Appl. Environ. Microbiol.* 70:3904–3909.

Cabelli, V., 1983, Public health and water quality significance of viral diseases transmitted by drinking water and recreational water. *Water Sci. Technol.* 15:1–15.

Cabelli, V. J., Dufour, A. P., McCabe, L. J., and Levin, M. A., 1982, Swimming-associated gastroenteritis and water quality. *Am. J. Epidemiol.* 115:606–616.

Carrington, E. G., Davis, R. D., and Pike, E. B., 1998a, Review of the scientific evidence relating to the controls on the agricultural use of sewage sludge. Part 1—the evidence underlying the 1989 Department of the Environment code of practice for agricultural use of sludge and the sludge (use in agriculture) regulations. WRC report no. DETR 4415/3. WRC Medmenham, Marlow, UK, p. 38.

Carrington, E. G., Davis, R. D., and Pike, E. B., 1998b, Review of the scientific evidence relating to the controls on the agricultural use of sewage sludge. Part 1—the evidence since 1989 relevant to controls on the agricultural use of sewage sludge. WRC report no. DETR 4415/3. WRC Medmenham, Marlow, UK, pp. 41–42.

Caul, E. O., 1994, Small round structured viruses: airborne transmission and hospital control. *Lancet* 343:1240–1242.

Chadwick, P. R., and McCann, R., 1994, Transmission of a small round structured virus by vomiting during a hospital outbreak of gastroenteritis. *J. Hosp. Infect.* 26:251–259.

Cheesbrough, J. S., Barkess-Jones, L., and Brown, D. W., 1997, Possible prolonged environmental survival of small round structured viruses. *J. Hosp. Infect.* 35:325–326.

Christensen, B. F., Lees, D., Wood, K. H., Bjergskov, T., and Green, J., 1998, Human enteric viruses in oysters causing a large outbreak of human food borne infection in 1996/97. *J. Shellfish Res.* 17:1633–1635.

Cliver, D. O., 1984, Significance of water and the environment in the transmission of virus disease. *Monogr. Virol.* 15:30–42.

Cliver, D. O., and Kostenbader, K. D., Jr., 1979, Antiviral effectiveness of grape juice. *J. Food Prot.* 42:100–104.

Cliver, D. O., and Riemann, H. P., 1999, *Foodborne Diseases*, 2nd ed., Academic Press, New York.

Cliver, D. O., Kostenbader, K. D., Jr., and Vallenas, M. R., 1970, Stability of viruses in low moisture foods. *J. Milk Food Tech.* 33:484–491.

Craun, G. F., 1988, Surface water supplies and health. *J. Am. Water Works Assoc.* 80:40–52.

Craun, G. F., Nwachuku, N., Calderon, R. L., and Craun, M. F., 2002, Outbreaks in drinking-water systems, 1991–1998. *J. Environ. Health* 65:16–25.

Croci, L., De Medici, D., Scaslfaro, C., Fiore, A., and Toti, L., 2002, The survival of hepatitis A virus in fresh produce. *Int. J. Food Microbiol.* 73:29–34.

D’Angelo, L. H., Hierholzer, J. C., Keenlyside, R. A., Anderson, L. J., and Mortone, W. J., 1979, Pharyngoconjunctival fever caused by adenovirus type 4: report of a swimming pool related outbreak with recovery of virus from pool water. *J. Infect. Dis.* 140:42–47.

De Flora, S., De Renzi, G., and Badolati, G., 1975, Detection of animal viruses in coastal seawater and sediments. *Appl. Environ. Microbiol.* 30:472–475.
Deng, M. Y., and Cliver, D. O., 1995, Persistence of inoculated hepatitis A virus in mixed human and animal wastes. Appl. Environ. Microbiol. 61:87–91.

Denis, F. A., Dupwis, T., Denis, N. A., and Brisou, J. L., 1977, Survie dans l’eau de mer de 20 souches de virus a ADN et ARN. J. Fr. Hydrol. 8:25–36.

Dimmock, N. L., 1967, Differences between the thermal inactivation of picornaviruses at high and low temperatures. Virology 31:338–353.

Divizia, M., De Filippis, P., Di Napoli, A., Venuti, A., Peres, B., and Pana, A., 1989a, Isolation of wild type hepatitis A virus from the environment. Water Res. 23:1155–1160.

Divizia, M., Santi, A. L., and Pana, A., 1989b, Ultrafiltration: an efficient second step for hepatitis A virus and poliovirus concentration. J. Virol. Methods 23:55–62.

Donaldson, A. I., and Ferris, N. P., 1976, The survival of some airborne animal viruses in relation to relative humidity. Vet. Microbiol. 1:413–420.

Doultree, J. C., Druce, J. D., Birch, C. J., Bowden, D. S., and Marshall, J. A., 1999, Inactivation of feline calicivirus, a Norwalk virus surrogate. J. Hosp. Infect. 41:51–57.

Elazhary, M. A., and Derbyshire, J. B., 1979, Effect of temperature, relative humidity and medium on the aerosol stability of infectious bovine rhinotracheitis virus. Can. J. Comp. Med. 43:158–167.

Enright, J. R., 1954, The epidemiology of paralytic poliomyelitis in Hawaii. Hawaii Med. J. 13:350–354.

Environmental Protection Agency, 1984, USEPA Manual Methods for Virology, US Environmental Protection Agency, Research and Development, 600/4-84-013. USEPA, Cincinnati, OH.

Fannin, K. F., Vana, S. T., and Jakubowski, W., 1985, Effect of activated sludge wastewater treatment plant on ambient air densities of aerosols containing bacteria and viruses. Appl. Environ. Microbiol. 49:1191–1196.

Farrah, S. R., Gerba, C. P., Wallis, C., and Melnick, J. L., 1976, Concentration of viruses from large volumes of tap water using pleated membrane filters. Appl. Environ. Microbiol. 31:221–226.

Farrah, S. R., Goyal, S. M., Gerba, C. P., Conklin, R. H., and Smith, E. M., 1978, Comparison between adsorption of poliovirus and rotavirus by aluminum hydroxide and activated sludge flocs. Appl. Environ. Microbiol. 35:360–363.

Farthing, M. J. G., 1989, Viruses and the Gut, Smith Kline & French, Garden City, UK.

Fattal, B., and Shuval, H. I., 1989, Epidemiological research on the relationship between microbial quality of coastal seawater and rotavirus induced gastroenteritis among bathers on the Mediterranean Israeli beaches. Research project no. ICP-CEH-039-ISR-16(D). WHO, Athens, pp. 1–25.

Fattal, B., Vasil, R. J., Katzenelson, E., and Shuval, H. I., 1983, Survival of bacterial indicator organisms and enteric viruses in the Mediterranean coastal waters of Tel-Aviv. Water Res. 17:397–402.

Fattal, B., Margalith, M., Shuval, H. I., Wax, Y., and Morag, A., 1987, Viral antibodies in agricultural populations exposed to aerosols from wastewater irrigation during a viral disease outbreak. Am. J. Epidemiol. 125:899–906.

Filippi, A., and Banwart, G. J., 1974, Effect of the fat content of ground beef on the heat inactivation of poliovirus. J. Food Sci. 39:865–868.

Finance, C., Brigaud, M., Lucena, F., Aymard, M., Bosch, A., and Schwartzbrod, L., 1982, Viral pollution of seawater at Barcelona. Zbl. Bakteriol. Mikrobiol. Hyg. B176:530–536.
Foy, H. M., Cooney, M. K., and Halten, J. B., 1968, Adenovirus type 3 epidemic associated with intermittent chlorination of a swimming pool. *Arch. Environ. Health* 17:795–802.

Fujioka, R. S., Loh, P. C., and Lau, L. S., 1980, Survival of human enteroviruses in the Hawaiian Ocean environment: evidence for virus inactivating microorganisms. *Appl. Environ. Microbiol.* 39:1105–1110.

Gajardo, R., Díez, J. M., Jofre, J., and Bosch, A., 1991, Adsorption-elution with negatively and positively-charged glass powder for the concentration of hepatitis A virus from water. *J. Virol. Methods* 31:345–352.

Gajardo, R., Bouchriti, N., Pintó, R. M., and Bosch, A., 1995, Genotyping of rotaviruses isolated from sewage. *Appl. Environ. Microbiol.* 61:3460–3462.

Gassilloud, B., Schwartzbrod, L., and Gantzer, B., 2003, Presence of viral genomes in mineral water: a sufficient condition to assume infectious risk? *Appl. Environ. Microbiol.* 69:3965–3969.

Gerba, C. P., and Schaiberger, G. E., 1975, Effect of particulates on virus survival in seawater, *J. Water Pollut. Cont. Fed.* 47:93–103.

Gerba, C. P., and Goyal, S. M., eds., 1982, *Methods in Environmental Virology*. Marcel Dekker, New York.

Gerba, C. P., Smith, E. M., and Melnick, J. L., 1977, Development of a quantitative method for detecting enteroviruses in estuarine sediments. *Appl. Environ. Microbiol.* 34:158–163.

Gerba, C. P., Goyal, S. M., Cech, I., and Bogdan, G. F., 1981, Quantitative assessment of the adsorptive behavior of viruses to soils. *Environ. Sci. Technol.* 15:940–944.

Gilgen, M., Germann, D., Lüthy, J., and Hübner, P., 1997, Three-step isolation method for sensitive detection of enterovirus, rotavirus, hepatitis A virus, and small round structured viruses in water samples. *Int. J. Food Microbiol.* 37:189–199.

Gill, O. N., Cubitt, W. D., McGwigan, D. A., Watney, B. M., and Bartlett, C. L. R., 1983, Epidemic of gastroenteritis caused by oysters contaminated with small round structured viruses. *Br. Med. J.* 287:1532–1534.

Gironés, R., Jofre, J., and Bosch, A., 1989, Natural inactivation of enteric viruses in seawater. *J. Environ. Qual.* 18:34–39.

Gironés, R., Jofre, J., and Bosch, A., 1990, Isolation of marine bacteria with antiviral properties. *Can. J. Microbiol.* 35:1015–1021.

Goh, K. T., Chan, L., Ding, J. L., and Oon C. J., 1984, An epidemic of cockles associated with hepatitis A in Singapore. *WHO Bull.* 62:893–897.

Goyal, S. M., and Gerba, C. P., 1979, Comparative adsorption of human enteroviruses, simian rotavirus, and selected bacteriophages to soils. *Appl. Environ. Microbiol.* 38:241–247.

Goyal, S. M., and Gerba, C. P., 1983, Viradel method for detection of rotavirus from seawater. *J. Virol. Methods* 7:279–285.

Goyal, S. M., Gerba, C. P., and Melnick, J. L., 1979, Human enteroviruses in oysters and their overlying waters. *Appl. Environ. Microbiol.* 37:572–581.

Grabow, W. O. K., Puttergill, D. L., and Bosch, A., 1993, Detection of adenovirus types 40 and 41 by means of the PLC/PRF/5 human liver cell line. *Water Sci. Tech.* 27:321–327.

Grausgruber, W., 1963, Investigations of the inactivation of infectious swine paralysis virus in scalded sausages. *Wiener Tierärztliche Monatsschrift* 50:678–685.

Gray, M., De León, R., Tepper, B. E., and Sobsey, M. D., 1993, Survival of hepatitis A virus (HAV), poliovirus and F-specific coliphages in disposable and landfill leachates. *Water Sci. Technol.* 27:429–432.
Green, J., Wright, P. A., Gallimore, C. I., Mitchell, O., Morgan-Capner, P., and Brown, D. W. G., 1998, The role of environmental contamination with small round structured viruses in a hospital outbreak investigated by reverse-transcriptase polymerase chain reaction assay. *J. Hosp. Infect.* 39:39–45.

Guix, S., Caballero, S., Villena, C., Bartolomé, R., Latorre, C., Rabella, N., Simó, M., Bosch, A., and Pintó, R. M., 2002, Molecular epidemiology of astrovirus infection in Barcelona, Spain. *J. Clin. Microbiol.* 40:133–139.

Gunderson, K., Brandberg, A., Magnusson, S., and Lycke, E., 1968, Characterization of a marine bacterium associated with virus inactivating capacity. *Acta Path. Microbiol. Scand.* 71:281–286.

Halliday, M. L., Kang, L. -Y., Zhou, T. -Z., Hu, M. -D., Pan, Q. -C., Fu, T. -Y., Huang, Y. S., and Hu, S. L., 1991, An epidemic of hepatitis A attributable to the ingestion of raw clams in Shanghai, China. *J. Infect. Dis.* 164:852–859.

Halvorsrud, J., and Orstavick, I., 1980. An epidemic of rotavirus-associated gastroenteritis in a nursing home for the elderly. *Scand. J. Infect. Dis.* 12:161–164.

Harper, G. J., 1961, Airborne microorganisms: survival tests with four viruses. *J. Hyg. (Cambridge)* 59:479–486.

Hejkal, T. W., Smith, E. M., and Gerba, C. P., 1984, Seasonal occurrence of rotavirus in sewage. *Appl. Environ. Microbiol.* 47:588–590.

Hemmes, J. H., Winkler, K. C., and Kool, S. M., 1960, Virus survival as a seasonal factor in influenza and poliomyelitis. *Nature* 188:430–438.

Hendley, J. O., Wenzel, R. P., and Gwaltney Jr, J. M., 1973, Transmission of rhinovirus colds by self-inoculation. *N. Engl. J. Med.* 288:1361–1364.

Henshilwood, K., Green, J., and Lees, D. N., 1998, Monitoring the marine environment for small round structured viruses (SRSVS): a new approach to combating the transmission of these viruses by molluscan shellfish. *Water Sci. Tech.* 38:51–56.

Hopkins, R. S., Gaspard, G. B., Williams, F. P. Jr, Karlin, R. J., Cukor, G., and Blacklow, N. R., 1984, A community waterborne gastroenteritis outbreak: evidence for rotavirus as the agent. *Am. J. Public Health* 74:263–265.

Huang, F. F., Haqshenas, G., Shivaprasad, H. L., Guenette, D. K., Woolcock, P. R., Larsen, C. T., Pierson, F. W., Elvinger, F., Toth, T. E., and Meng, X. J., 2002, Heterogeneity and seroprevalence of a newly identified avian hepatitis E virus from chickens in the United States. *J. Clin. Microbiol.* 40:4197–4202.

Huber, M. S., Gerba, C. P., Abbaszedagan, M., Robinson, J. A., and Bradford, S. M., 1994, Study of persistence of enteric viruses in land filled disposable diapers. *Environ. Sci. Technol.* 28:1767–1772.

Hugues, B., Cini, A., Plissier, M., and Lefebre, J. R., 1980, Recherche des virus dans le milieu marin à partir d’échantillons de volumes différents. *Eau. Ouetec.* 13:199–203.

Humphrey, T. J., Cruickshank, J. G., and Cubitt, W. D., 1984, An outbreak of calicivirus associated gastroenteritis in an elderly persons home: a possible zoonosis? *J. Hyg. (London)* 93:293–299.

Hung, T., Wang, C. H., Fang, Z., Chou, Z., Chang, X., Liong, X., Chen, G., Yao, H., Chaoon T., Ye, W., Den, S., and Chang, W., 1984, Waterborne outbreak of rotavirus diarrhoea in adults in China caused by a novel rotavirus. *Lancet* 2:1139–1142.

Hurst, C. J., 1987, Influence of aerobic microorganisms upon virus survival in soil. *Can. J. Microbiol.* 34:696–699.

Hurst, C. J., Farrah, S. R., Gerba, C. P., and Melnick, J. L., 1978, Development of quantitative methods for the detection of enteroviruses in sewage sludges during activation and following land disposal. *Appl. Environ. Microbiol.* 36:81–89.
Hurst, C. J., Gerba, C. P., and Cech, I., 1980, Effects of environmental variables and soil characteristics on virus survival in soil. *Appl. Environ. Microbiol.* 40:1067–1079.

Ijaz, M. K., Brunner, A. H., Sattar, S. A., Nair, R. C., and Johnson-Lussenburg, C. M., 1985a, Survival characteristics of airborne human coronavirus 229E. *J. Gen. Virol.* 66:2743–2748.

Ijaz, M. K., Sattar, S. A., Johnson-Lussenburg, C. M., and Springthorpe, V. S., 1985b, Comparison of the airborne survival of calf rotavirus and poliovirus type 1 (Sabin) aerosolized as a mixture, *Appl. Environ. Microbiol.* 49:289–293.

Jiang, X., Espul, C., Zhong, W. M., Cuello, H., and Matson, D. O., 1999, Characterization of a novel human calicivirus that may be a naturally occurring recombinant. *Arch. Virol.* 144:2377–2387.

Jofre, J., Blasi, M., Bosch, A., and Lucena, F., 1989, Occurrence of bacteriophages infecting *Bacteroides fragilis* and other viruses in polluted marine sediments. *Water Sci. Tech.* 21:15–19.

Kaplan, J. E., Godman, R. A., Schoenberger, L. B., Lippy, E. C., and Gary, W., 1982, Gastroenteritis due to Norwalk virus: an outbreak associated with a municipal water system. *J. Infect. Dis.* 146:190–197.

Kapuscinski, R. B., and Mitchell, R., 1980, Processes controlling virus inactivation in coastal waters. *Water Res.* 14:363–371.

Katzenelson, E., 1978, Survival of viruses, in: *Indicators of Viruses in Water and Food* (G. Berg, eds.), Ann Arbor Sci., Ann Arbor, MI, pp. 39–50.

Katzenelson, E., Fattal, B., and Hostovesky, T., 1976, Organic flocculation: an efficient second-step concentration method for the detection of viruses in tap water. *Appl. Environ. Microbiol.* 32:638–639.

Keswick, B. H., Gerba, C. P., DuPont, H. L., and Rose J. B., 1984, Detection of enteroviruses in treated drinking water. *Appl. Environ. Microbiol.* 47:1290–1294.

Keswick, B. H., Pickering, L. K., DuPont, H. L., and Woodward, W. E., 1983, Survival and detection of rotaviruses on environmental surfaces in day care centers. *Appl. Environ. Microbiol.* 46:813–816.

Khuroo, M. S., 1980, Study of an epidemic of non A, non B hepatitis—possibility of another human hepatitis virus distinct from post-transfusion non A, non B type. *Am. J. Med.* 68:818–824.

Kingsley, D. H., Meade, G. K., and Richards, G. P., 2002, Detection of both hepatitis A virus and Norwalk-like virus in imported clams associated with food-borne illness. *Appl. Environ. Microbiol.* 68:3914–3918.

Kohn, A., Gitelman, J., and Inbar, M., 1980, Unsaturated free fatty acids inactivate animal enveloped viruses. *Arch. Virol.* 66:301–307.

Konowalchuk, J., and Speirs, J. I., 1975, Survival of enteric viruses on fresh vegetables. *J. Milk Food Technol.* 38:469–472.

Konowalchuk, J., and Speirs, J. I., 1976, Virus inactivation by grapes and wines. *Appl. Environ. Microbiol.* 32:757–763.

Konowalchuk, J., and Speirs, J. I., 1978, Antiviral effect of commercial juices and beverages. *Appl. Environ. Microbiol.* 35:1219–1220.

Koopman, J. S., Eckert, E. A., Greenberg, H. B., Strohm, B. C., Isaacson, R. E., and Monto, A. S., 1982, Norwalk virus enteric illness acquired by swimming exposure. *Am. J. Epidemiol.* 115:173–177.

Kopecka, H., Dubrou, S., Prévot, J., Maréchal, J., and López-Pila, J. M., 1993, Detection of naturally occurring enteroviruses in waters by reverse transcription, polymerase chain reaction and hybridization. *Appl. Environ. Microbiol.* 59:1213–1219.
Kott, H., and Fishelson, L., 1974, Survival of enteroviruses on vegetables irrigated with chlorinated oxidation pond effluents. *Isr. J. Technol.* 12:290–297.

Ksiazek, T. G., Erdman, D., Goldsmith, C. S., Zaki, S. R., Peret, T., Emery, S., Tong, S., Urbani, C., Comer, J. A., Lim, W., Rollin, P. E., Dowell, S. F., Ling, A.-E., Humphrey, C. D., Shieh, W.-J., Guarnier, J., Paddock, C. D., Rota, P., Fields, B., DeRisi, J., Yang, J.-Y., Cox, N., Hughes, J. M., LeDuc, J. W., Bellini, W. J., Anderson, L. J., and the SARS Working Group, 2003, A novel coronavirus associated with severe acute respiratory syndrome. *N. Engl. J. Med.* 348: 1953–1966.

Kukkula, M., Maunula, L., Silvennoinen, E., and von Bosndorff, C. H., 1999, Outbreak of viral gastroenteritis due to drinking water contaminated by Norwalk-like viruses. *J. Infect. Dis.* 180:1771–1776.

La Belle, R. L., Gerba, C. P., Goyal, S. M., Melnick, J. L., Lech, I., and Bogdan, G. F., 1980, Relationships between environmental factors, bacterial indicators and the occurrence of enteric viruses in estuarine sediments. *Appl. Environ. Microbiol.* 39:588–596.

Landry, E. F., Vaughn, J. M., Vicale, T. J., and Mann, R., 1983, Accumulation of sediment-associated viruses in shellfish. *Appl. Environ. Microbiol.* 45:238–247.

Larkin, E. P., Tierney, J. T., and Sullivan, R., 1976, Persistence of virus on sewage-irrigated vegetables. *J. Environ. Eng. Div.* 1:29–35.

Le Bris, J. M., Billaudel, S., Bertrand, P., Loukou, G., and et Courtieu, A. L., 1983, Recherche des virus et des salmonelles dans la Loire par une méthode d’adsorption-élution sur filtres en microfibre de verre. *Tech. Sci. Munic. L’Eau.* 6:303–306.

Le Guyader, F., Dubois, E., Menard, D., and Pommepuy, M., 1994, Detection of hepatitis A virus, rotavirus, and enterovirus in naturally contaminated shellfish and sediment by reverse transcription-semested PCR. *Appl. Environ. Microbiol.* 60:3665–3671.

Le Guyader, F., Neill, F. H., Estes, M. K., Monroe, S. S., Ando, T., and Atmar, R. L., 1996, Detection and analysis of a SRSV strain in oysters implicated in an outbreak. *Appl. Environ. Microbiol.* 62:4268–4272.

Lemon, S. M., 1985, Type A viral hepatitis—new developments in an old disease. *N. Engl. J. Med.* 313:1059–1067.

Lenaway, D. D., Brockmann, R., Dolan, G. J., and Cruz-Uribe F., 1989, An outbreak of an enterovirus-like illness at a community wading pool: implications for public health inspection programs. *Am. J. Public Health* 79:889–890.

Lewis, G. D., and Metcalf, T. G., 1988, Polyethylene glycol precipitation for recovery of pathogenic viruses, including hepatitis A virus and human rotavirus, from oysters, water and sediment samples. *Appl. Environ. Microbiol.* 54:1983–1988.

Linco, S. J., and Grohmann, G. S., 1980, The Darwin outbreak of oyster associated viral gastroenteritis. *Med. J. Aust.* 1:211–213.

Lippy, E. C., and Waltrip, S. C., 1984, Waterborne disease outbreaks 1946–1980: a thirty-five-year perspective. *J. Am. Water Works Assoc.* 76:60–67.

Lo, S., Gilbert, J., and Hetrick, F., 1976, Stability of human enteroviruses in estuarine and marine waters. *Appl. Environ. Microbiol.* 32:245–248.

Lopman, B. A., Reacher, M. H., Van Duijnhoven, Y., Hanon, F. X., Brown, D., and Koopmans, M., 2003, Viral gastroenteritis outbreaks in Europe, 1995–2000. *Emerg. Infect. Dis.* 9:90–96.
Lucena, F., Bosch, A., Jofre, J., and Schwartzbrod, L., 1985, Identification of viruses isolated from sewage, river water and coastal seawater in Barcelona. *Water Res.* 19:1237–1239.

Mahl, M. C., and Sadler, C., 1975, Virus survival on inanimate surfaces. *Can. J. Microbiol.* 21:819–823.

Matossian, A. M., and Garabedian, G. A., 1967, Virucidal action of seawater. *Am. J. Epidemiol.* 85:1–8.

Mbithi, J. N., Springthorpe, V. S., and Sattar, S. A., 1991, Effect of relative humidity and air temperature on survival of hepatitis A virus on environmental surfaces. *Appl. Environ. Microbiol.* 59:3463–3469.

Mbithi, J. N., Springthorpe, V. S., Boulet, J. R., and Sattar, S. A., 1992, Survival of hepatitis A virus on human hands and its transfer on contact with animate and inanimate surfaces. *J. Clin. Microbiol.* 30:757–763.

McGeady, M. L., Siak, J. S., and Crowell, R. L., 1979, Survival of coxsackie virus B3 under diverse environmental conditions. *Appl. Environ. Microbiol.* 37:972–977.

McNulty, M. S., 1978, Rotaviruses. *J. Gen. Virol.* 40:1–18.

Mead, P. S., Slutsker, L., and Dietz, V., 1999, Food-related illness and death in the United States. *Emerg. Inf. Dis.* 5:607–625.

Melnick, J. U., Stewien, K. E., Hársi, C. M., Queiroz, A. P. S., Candeias, J. M. G., and Candeias, J. A. N., 1997, Detection of rotavirus in sewage and creek water: efficiency of the concentration method. *Mem. Inst. Oswaldo Cruz* 92:97–100.

Melnick, J. L., 1957, A water-borne urban epidemics of hepatitis, in: *Hepatitis: Frontiers* (G. A. LoGrippo, F. W. Hartman, J. G. Mateer, and J. Barron, eds.), Little Brown, Boston, pp. 211–225.

Melnick, J. L., and Gerba, C. P., 1980, The ecology of enteroviruses in natural waters. *Crit. Rev. Environ. Control.* 10:65–93.

Meng, X. J., Purcell, R. H., Halbur, P. G., Lehman, J. R., Webb, D. M., Tsareva, T. S., Haynes, J. S., Thacker, B. J., and Emerson S. U., 1997, A novel virus in swine is closely related to the human hepatitis E virus. *Proc. Natl. Acad. Sci. U. S. A.* 94:9860–9865.

Miller, W. S., and Artenstein, M. S., 1967, Aerosol stability of three acute respiratory disease viruses. *Proc. Soc. Exp. Biol. Med.* 125:222–227.

MMWR (Morbidity and Mortality Weekly Report), 2002, Surveillance for waterborne-disease outbreaks—United States, 1999–2000. *MMWR* 51:1–52.

MMWR (Morbidity and Mortality Weekly Report), 2003, Hepatitis A outbreak associated with green onions at a restaurant—Monaca, Pennsylvania, 2003. *MMWR* 52:1155–1157.

Moe, K., and Harper, G. J., 1983, The effect of relative humidity and temperature on the survival of bovine rotavirus in aerosol. *Arch. Virol.* 76:211–216.

Moe, K., and Shirley, J. A., 1982, The effects of relative humidity and temperature on the survival of human rotavirus in feces. *Arch. Virol.* 72:179–186.

Moore, M., 1982, Enteroviral disease in the United States, 1970–1979. *J. Infect. Dis.* 146:103–108.

Moore, A. C., Herwaldt, B. L., Craun, G. F., Calderon, R. L., Highsmith, A. K., and Juranek, D. D., 1994, Waterborne disease in the United States, 1991 and 1992. *J. Am. Water Works Assoc.* 86:87–99.

Morse, D. L., Guzewich, J. J., Hanrahan, J. P., Stricof, R., Shayegani, M., Deibel, R., Grabau, J. C., Nowak, N. A., Herrmann, J. E., Cukor, G., and Blacklow, N. R., 1986, Widespread outbreaks of clam- and oyster-associated gastroenteritis: role of Norwalk virus. *New Engl. J. Med.* 314:678–681.
Mosley, J. W., 1967, Transmission of viruses by drinking water, in: Transmission of Viruses by the Water Route (G. Berg, eds.), John Wiley & Sons, New York, pp. 5–23.

Mounts, A. W., Ando, T. A., Koopmans, M., Bresee, J. S., Inouye, S., Noel, J., and Glass, R. I., 2000, Cold weather seasonality of gastroenteritis associated with Norwalk-like viruses. *J. Infect. Dis.* 181:284–287.

Murphy, A. M., Grohmann, G. S., Christopher, R. J., Lopez, W. A., Davey, G. R., and Millsom, R. H., 1979, An Australia-wide outbreak of gastroenteritis from oysters caused by Norwalk virus. *Med. J. Aust.* 2:329–333.

Murphy, A. M., Grohmann, G. S., and Sexton, F. H., 1983, Infectious gastroenteritis in Norfolk Island and recovery of viruses from drinking water. *J. Hyg.* 91:139–146.

Muscillo, M., La Rosa, G., Carducci, A., Cantiani, L., and Marianelli, C., 1997, Molecular analysis of poliovirus 3 isolated from an aerosol generated by a waste water treatment plant. *Water Res.* 12:3125–3131.

Nuanualsuwan, S., and Cliver, D. O., 2002, Pretreatment to avoid positive RT-PCR results with inactivated viruses. *J. Virol. Methods* 104:217–225.

O’Brien, R. T., and Newman, J. S., 1977, Inactivation of poliovirus and coxsackie viruses in surface water. *Appl. Environ. Microbiol.* 33:334–340.

Oh, D.-Y., Gaedicke, G., and Schreir, J. M., 2003, Viral agents of acute gastroenteritis in German children: prevalence and molecular diversity. *J. Med. Virol.* 71:82–93.

O’Mahony, M., Gooch, C. D., Smyth, D. A., Thrussell, A. J., Bartlett, C. L. R., and Noah, N. D., 1983, Epidemic hepatitis A from cockles. *Lancet* I:518–520.

Parashar, U. D., Holman, R. C., Clarke, M. J., Bresee, J. S., and Glass R. I., 1998, Hospitalizations associated with rotavirus diarrhea in the United States, 1993 through 1995: surveillance based on the new ICD-9-CM rotavirus-specific diagnostic code. *J. Infect. Dis.* 177:13–17.

Payment, P., Berte, A., Prevost, M., Ménard, B., and Barbeau, B., 2000, Occurrence of pathogenic microorganisms in the Saint-Lawrence river (Canada) and comparison of health risks for populations using it as their source of drinking water. *Can. J. Microbiol.* 46:565–576.

Pfirrmann, A., and Bossche, G. V., 1994, Occurrence and isolation of airborne human enteroviruses from waste disposal and utilization plants. *Zbl. Hyg.* 196:38–51.

Pintó, R. M., Diez, J. M., and Bosch, A., 1994, Use of the colonic carcinoma cell line CaCo-2 for in vivo amplification and detection of enteric viruses. *J. Med. Virol.* 44:310–315.

Pintó, R. M., Gajardo, R., Abad, F. X., and Bosch, A., 1995, Detection of fastidious infectious enteric viruses in water. *Environ. Sci. Tech.* 29:2636–2638.

Pintó, R. M., Villena, C., Le Guyader, F., Guix, S., Caballero, S., Pompeuy, M., and Bosch, A., 2001, Astrovirus detection in wastewater samples. *Water Sci. Tech.* 42:73–76.

Ramalingaswami, V., and Purcell, R. H., 1988, Waterborne non-A, non-B hepatitis. *Lancet.* 1:571–573.

Rao, V. C., and Melnick, J. L., 1986, Environmental virology, in: *Aspects of Microbiology 13* (J. A. Cole, C. J. Knowles, and D. Schlessinger, eds.), American Society for Microbiology, Washington, DC.

Rao, V. C., Seidel, K. N., Goyal, S. M., Metcalf, T. C., and Melnick, J. L., 1984, Isolation of enteroviruses from water, suspended solids and sediments from Galveston bay; survival of poliovirus and rotavirus adsorbed to sediments. *Appl. Environ. Microbiol.* 48:404–409.
Rao, V. C., Metcalf, T. G., and Melnick, J. L., 1986, Development of a method for concentration of rotavirus and its application to recovery of rotaviruses from estuarine waters. *Appl. Environ. Microbiol.* 52:484–488.

Raphael, R. A., Sattar, S. A., and Springthorpe, V. S., 1985, Long term survival of human rotavirus in raw and treated river water. *Can. J. Microbiol.* 31:124–128.

Reagan, K. J., McGeady, M. L., and Crowell, R. L., Persistence of human rhinovirus infectivity under diverse environmental conditions. *Appl. Environ. Microbiol.* 41:618–627.

Redman, J. A., Grant, S. B., Olson, T. M., Hardy, M. E., and Estes, M. K., 1997, Filtration of recombinant Norwalk Virus particles and bacteriophage MS2 in quartz sand: importance of electrostatic interactions. *Environ. Sci. Tech.* 31:3378–3383.

Reid, T. M. S., and Robinson, H. G., 1987, Frozen raspberries and hepatitis A. *Epidemiol. Infect.* 98:109–112.

Reyes, G. R., 1993, Hepatitis E virus (HEV): molecular biology and emerging epidemiology. *Prog. Liver Dis.* 11:203–213.

Reynolds, K. A., Gerba, C. P., and Pepper, I. L., 1996, Detection of infectious enteroviruses by an integrated cell culture-PCR procedure. *Appl. Environ. Microbiol.* 62:1424–1427.

Reynolds, K. A., Gerba, C. P., Abbasazdegan, M., and Pepper, I. L., 2001, ICC/PCR detection of enteroviruses and hepatitis A virus in environmental samples. *Can. J. Microbiol.* 47:153–157.

Richards, G. P., 1985, Outbreaks of shellfish-associated enteric illness in the United-States: requisite for development of viral guidelines. *J. Food Prot.* 48:815–823.

Rocchi, G., Vella, S., Resta, S., Cochi, S., Donelli, G., Tangucci, F., Manichella, D., Varveri, A., and Inglese R., 1981, Outbreak of rotavirus gastroenteritis among premature infants. *Br. Med. J.* 283:886.

Ryder, R. W., McGowan, J. E., Hatch, M. H., and Palmer, E. L., 1977, Reovirus-like agent as a cause of nosocomial diarrhoea in infants. *J. Pediatr.* 90:698–702.

Rzetka, A., and Cook, N., 2004, Survival of human enteric viruses in the environment and food. *FEMS Microbiol. Rev.* (in press).

Salo, R. J., and Cliver, D. O., 1976, Effect of acid pH, salt and temperature on the infectivity and physical integrity of enteroviruses. *Arch. Virol.* 52:269–282.

Sánchez, G., Bosch, A., and Pintó, R. M., 2003, Genome variability and capsid structural constraints of hepatitis A virus. *J. Virol.* 77:452–459.

Santamaría, J., and Toranzos, G. A., 2003, Enteric pathogens and soil: a short review. *Int. Microbiol.* 6:5–9.

Sarrette, B. A., Danglot, C. D., and Vilagines, R., 1977, A new and simple method for recuperation of enterovirus from water. *Water Res.* 11:355–358.

Sattar, S. A., Ijaz, M. K., Johnson-Lussenburg, C. M., and Springthorpe, V. S., 1984, Effect of relative humidity on the airborne survival of rotavirus SA11. *Appl. Environ. Microbiol.* 47:879–881.

Sattar, S. A., Lloyd-Evans, N., Springthorpe, V. S., and Nair, R. C., 1986, Institutional outbreaks of rotavirus diarrhoea: potential role of fomites and environmental surfaces as vehicles for virus transmission. *J. Hyg. Camb.* 96:277–289.

Sattar, S. A., Karim, Y. G., Springthorpe, V. S., and Johnson-Lussenburg, C. M., 1987, Survival of human rhinovirus type 14 dried onto nonporous inanimate surfaces: effect of relative humidity and suspending medium. *Can. J. Microbiol.* 33:802–806.

Sattar, S. A., Dimock, K. D., Ansari, S. A., and Springthorpe, V. S., 1988, Spread of acute hemorrhagic conjunctivitis due to enterovirus 70: effect of air temperature and relative humidity on virus survival on fomites. *J. Med. Virol.* 25:289–296.
Schaiberger, G. E., Edmond, T. D., and Gerba, C. P., 1982, Distribution of enteroviruses in sediments contiguous with a deep marine sewage outfall. *Water Res.* 16:1425–1428.

Schwartzbrod, L., and Lucena, F., 1978, Concentration des enterovirus dans les eaux par adsorption sur poudre de verre: proposition d’un appareillage simplifié. *Microbio*. 4:55–68.

Shields, P. A., and Farrah, S. R., 1986, Concentration of viruses in beef extract by flocculation with ammonium sulphate. *Appl. Environ. Microbiol.* 51:211–213.

Shirai, J., Shimizu, M., and Fukusho, A., 1985, Coronavirus-, calicivirus-, and astrovirus-like particles associated with acute porcine gastroenteritis. *Nippon Juigaku Zasshi* 47:1023–1026.

Shuval, H. I., Thompson, A., Fattal, B., Cymbalista, S., and Weiner, Y., 1971, Natural virus inactivation processes in seawater. *J. San. Eng. Div. Am. Soc. Civ. Eng.* 5:587–600.

Sobsey, M. D., and Jones, B. L., 1979, Concentration of poliovirus from tap water using positively charged microporous filters. *Appl. Environ. Microbiol.* 37:588–595.

Sobsey, M. D., and Glass, J. S., 1984, Influence of water quality on enteric virus concentration by microporous filter methods. *Appl. Environ. Microbiol.* 47:956–960.

Sobsey, M. D., and Hickey, A. R., 1985, Effect of humic and fulvic acid on poliovirus concentration from water by microporous filtration. *Appl. Environ. Microbiol.* 49:259–264.

Sobsey, M. D., Dean, C. H., Knuckles, M. E., and Wagner, R. A., 1980, Interactions and survival of enteric viruses in soil materials. *Appl. Environ. Microbiol.* 40:92–101.

Sobsey, M. D., Shields, P. A., Hauchman, F. S., Davis, A. L., Rullman, V. A., and Bosch, A., 1988, Survival and persistence of hepatitis A virus in environmental samples, in: *Viral Hepatitis and Liver Disease* (A. Zuckerman, eds.), Alan R. Liss, New York, pp. 121–124.

Sobsey, M. D., Shields, P. A., Hauchman, F. H., Hazard, R. L., and Caton, III, L. W., 1989, Survival and transport of hepatitis A virus in soils, groundwater and wastewater. *Water Sci. Technol.* 10:97–106.

Steinman, J., 1981, Detection of rotavirus in sewage. *Appl. Environ. Microbiol.* 41:1043–1045.

Straub, T. M., Pepper, I. L., and Gerba, C. P., 1992, Persistence of viruses in desert soils amended with anaerobically digested sewage sludge. *Appl. Environ. Microbiol.* 58:636–641.

Straub, T. M., Pepper, I. L., and Gerba, C. P., 1993, Virus survival in sewage sludge amended desert soil. *Water Sci. Tech.* 27:421–424.

Strauss, M., 1994, Health implications of excreta and wastewater use—Hubei environmental sanitation study, 2nd workshop, Hubei, Wuhan.

Teltsch, B., Kedmi, S., Bonnet, L., Borenstajn-Rotem, Y., and Katzenelson, E., 1980, Isolation and identification of pathogenic microorganisms at wastewater-irrigated fields: ratios in air and wastewater. *Appl. Environ. Microbiol.* 39:1183–1190.

Thurston-Enriquez, J. A., Haas, C. N., Jacangelo, J., and Gerba, C. P., 2003a, Chlorine inactivation of adenovirus type 40 and feline calicivirus. *Appl. Environ. Microbiol.* 69:3979–3985.

Thurston-Enriquez, J. A., Haas, C. N., Jacangelo, J., Riley, K., and Gerba, C. P., 2003b, Inactivation of feline calicivirus and adenovirus type 40 by UV radiation. *Appl. Environ. Microbiol.* 69:577–582.

Tierney, J. T., Sullivan, R., and Larkin, E. P., 1977, Persistence of poliovirus 1 in soil and on vegetables grown in soil previously flooded with inoculated sewage sludge or effluent. *Appl. Environ. Microbiol.* 33:109–113.
Toranzo, A. E., Barja, J. L., and Hetrick, F. M., 1983, Mechanism of poliovirus inactivation by cell-free filtrates of marine bacteria. *Can. J. Microbiol.* 29:1481–1486.

Trouwborst, T., Kuyper, S., de Jong, J. C., and Plantinga, A. D., 1974, Inactivation of some bacterial and animal viruses by exposure to liquid-air interfaces. *J. Gen. Virol.* 24:155–165.

Tsai, Y.-L., Tran, B., and Palmer, C. J., 1995, Analysis of viral RNA persistence in seawater by reverse transcriptase-PCR. *Appl. Environ. Microbiol.* 61:363–366.

Tsang, T., 2003, Environmental issues. WHO Global Conference on Severe Acute Respiratory Syndrome (SARS), Kuala Lumpur, Malaysia, 17–18 June 2003.

Unicomb, L. E., Podder, G., Gentsch, J. R., Woods P. A., Hasan, K. Z., Faruque, A. S., Albert, M. J., and Glass, R. I., 1999, Evidence of high-frequency genomic reassortment of group A rotavirus strains in Bangladesh: emergence of type G9 in 1995. *J. Clin. Microbiol.* 37:1885–1891.

van der Poel, W. H. M., Verschoor, F., van der Heide, R., Herrera, M. I., Vivo, A., Kooreman M., and de Roda Husman, A. M., 2001, Hepatitis E virus sequences in swine related to sequences in humans, the Netherlands. *Emerg. Infect. Dis.* 6:970–976.

Vaughn, J. M., Landry, E. F., Thomas, M. Z., Vicale, T. J., and Penello, W. F., 1979, Survey of human enterovirus occurrence in fresh and marine surface waters on Long-Island. *Appl. Environ. Microbiol.* 38:290–296.

Vilaginès, P., Sarrette, B., Champsaur, H., Hugues, B., Dubrou, S., Joret, J.-C., Laveran, H., Lesne, J., Paquin, J. L., Delattre, J. M., Oger, C., Alame, J., Grateloup, I., Perrollot, H., Sercau, R., Sinégre, F., and Vilaginès, R., 1997, Round robin investigation of glass wool method for poliovirus recovery from drinking water and sea water. *Water Sci. Tech.* 35:445–449.

Villena, C., Gabrielli, R., Pintó, R. M., Guix, S., Donia, D., Buonomo, E., Palombi, L., Cenko, F., Bino, S., Bosch, A., and Divizia, M., 2003, A large infantile gastroenteritis outbreak in Albania caused by multiple emerging rotavirus genotypes. *Epidemiol. Infect.* 131:1105–1110.

Wadell, G., Allard, A. Svensson, L., and Uhnoo, I., 1989, Enteric adenoviruses, in: *Viruses and the Gut* (M. J. G. Farthing, eds.), Smith, Kline and French, Welwyn Garden City, UK., pp. 70–78.

Wait, D. A., and Sobsey, M. D., 1983, Method for recovery of enteric viruses from estuarine sediments with caotropic agents. *Appl. Environ. Microbiol.* 46:379–385.

Walter, R., Macht, W., Durkop, J., Becht, R., Hornig, U., and Schulze, P., 1989, Virus levels in river waters. *Water Res.* 21:133–138.

Ward, B. K., and Irving, L. G., 1987, Virus survival on vegetables spray-irrigated with wastewater. *Water Res.* 21:57–63.

Ward, B. K., Chenoweth, C. M., and Irving, L. G., 1982, Recovery of viruses from vegetable surfaces. *Appl. Environ. Microbiol.* 44:1389–1394.

Ward, R. L., Knowlton, D. R., and Winston, P. E., 1986, Mechanism of inactivation of enteric viruses in fresh water. *Appl. Environ. Microbiol.* 52:450–459.

Weissman, J. B., Craun, G. F., Lawrence, D. N., Pollard, R. A., Saslaw, M. S., and Gargarosa, E. J., 1976, An epidemic of gastroenteritis traced to a contaminated public water supply. *Am. J. Epidemiol.* 103:391–398.

Yeager, J. G., and O’Brien, R. T., 1979, Enterovirus inactivation in soil. *Appl. Environ. Microbiol.* 38:694–701.