6

Survival of Viruses in the Marine Environment

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6.1. INTRODUCTION

It has been well established that human pathogenic viruses may be transmitted through the marine environment due to the release of sewage by polluted rivers, outfalls, or release from vessels. These wastes contain human enteric viruses, which if ingested, or in some cases inhaled, can cause a wide variety of illnesses. Their ability to be transmitted by this route is because of their capability to remain infectious long enough in the marine environment to come in contact with a susceptible host. Transmission routes may be fairly direct, such as ingested of contaminated seawater by a swimmer, or more complex by prolonged survival in sediments which are later resuspended and accumulated in shellfish during feeding. The virus is then transmitted during consumption of the shellfish. To understand the potential for human enteric virus transmission through the marine environment numerous studies have been conducted on factors which influence their persistence in this environment (Table 6.1). This review focuses on factors that could play a role in the survival of human pathogenic viruses in the marine environment.

6.2. FACTORS AFFECTING VIRUS SURVIVAL IN MARINE WATER

6.2.1. Types of Viruses

All of the human enteric viruses transmitted by water and food are composed only of a nucleic acid genome of either RNA or DNA enclosed in a protective protein coat. There are currently over 140 different human enteric viruses that are known. Most of these have a genome of a single strand of RNA (sRNA), e.g. enteroviruses, astroviruses, caliciviruses, hepatitis A, hepatitis E. Rotaviruses have a double strand of RNA (dsRNA), and adenoviruses a double strain of DNA (dsDNA). The rotaviruses and adenoviruses are about twice the size (60 to 70 nm) of the sRNA viruses. Coronaviruses, picobirnavirues, and parvoviruses have been associated with gastroenteritis transmission but transmission by water or food has not been clearly demonstrated. Coronavirus (sRNA) are the only human enteric viruses with a lipid coat. The recently discovered picobirnavirues are small double stranded RNA viruses associated with

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Table 6.1. Factors affecting virus survival in natural waters.

Factor	Effect	Remarks
Temperature	Protein or nucleic acid denaturation	Temperature is probably the most important factor in virus survival; viruses survive longer at lower temperatures
Light	Configuration change or breakage of the nucleic acid	UV in sunlight and natural photodynamic processes may act upon the virus in natural waters
pH	Release of nucleic acid or disassembly of capsid	Most enteric viruses are stable at pH values found in most natural waters
Salts	Stabilization of capsid structure	Some viruses are protected against heat inactivation by the presence of certain cations; the reverse is also true
Proteinaceous material	Protection from enzymes and sunlight?	Viruses survive longer in the presence of sewage and other soluble organic material
Suspended particulate matter; sediments	Thermostabilization of protein capsid; protection from the action of enzymes	Virus survival is prolonged by adsorption to clays and other suspended solids, viruses may also be inactivated by adsorption to other solid surfaces
Biological factors	Enzymes; adsorption; nonenzymatic interaction affecting capsid structure	Certain viruses are inactivated more readily in the presence of aquatic bacteria; can also act to protect virus by adsorption to surface or other factors

diarrhea in immunocomprimised persons and travelers to developing countries. Parvoviruses are small (18 to 25 nm) singled stranded DNA viruses. They are among the most thermally resistant viruses known.

Most of our understanding on virus survival in the marine environment before 1990 comes from studies with enteroviruses (sRNA) and bacteriophages of *Escherichia coli* (coliphages), although hepatitis A and noroviruses (a calicivirus) have been the viruses usually associated with transmission through the marine environment. This is because noroviruses have not yet been grown in the laboratory. During the last decade a great deal of information has been gained about the ecology and survival of phages of marine microorganisms (Wommack & Colwell, 2000). This has provided new information on potential mechanisms of virus persistence that may be useful in understanding the survival of human pathogenic viruses. However, it must be remembered that human enteric viruses are not native to this environment and the relative importance of factors affecting inactivation may be different between the two groups of viruses.

6.2.2. Temperature

While many factors are involved in the survival of viruses in natural waters, temperature probably plays the most decisive role. Temperature is the only well-defined factor with a consistent effect on virus survival. At freezing or near-freezing temperatures, viruses may remain infectious for many months (Lo et al., 1976). Compared to many other viral groups that infect man, enteric viruses are usually heat stable. Hepatitis A virus, adenoviruses and parvoviruses appear to be the most thermostable enteric viruses (Crance et al., 1998; Brauniger et al., 2000; Gerba, unpublished observations).

In addition, viral populations are heterogeneous and include thermo-resistant mutants (Pohjanpelto, 1961). The effect of temperature on the rate of viral inactivation is also related to factors such as pH, salts, organics, and the presence of particulate matter (Pohjanpelto, 1961; Salo & Cliver, 1976; Wallis et al., 1965; Wallis & Melnick, 1962). Certain cations in solution appear to protect some viruses from thermoinactivation, while having the opposite effect on other viruses. Thus, rotavirus SA-11 is more rapidly inactivated by heating at 50°C in 2 M $MgCl_2$, while reovirus type 1 infectivity is stabilized (Estes et al., 1979). In contrast, SA-11 infectivity is stabilized at 50°C in the presence of 2 M $MgSO_4$. At high temperatures, poliovirus type 1 is more stable in seawater than in distilled water (Liew & Gerba, 1980), indicating that salts in natural waters may have some stabilizing effect on virus survival.

Poliovirus type 1 has been shown to be thermostablized by association with sediments (Liew & Gerba, 1980). At 50°C, poliovirus type 1 in artificial seawater is protected from inactivation in the presence of marine sediment. Almost all of the virus remained adsorbed to the sediment. The virus was also protected against inactivation at 24 and 37°C.

Virus inactivation at elevated temperatures may result primarily from protein denaturation. Poliovirus RNA becomes more susceptible to ribionuclease after it is heated at high temperatures, indicating structural alterations in the viral capsid (Dimmock, 1967). At lower temperatures, inactivation due to damage of the RNA may also be important.

6.2.3. pH

At the pH of most natural waters (pH 5 to 9), enteric viruses are very stable. Most enteric viruses are generally more stable at a pH between 3 to 5 than at alkaline pH (9 to 12). Adenoviruses and rotaviruses are readily inactivated at pH 10, while enteroviruses can survive at a pH of 11 to 11.5 for short periods of time (Gerba & Goyal, 1982). The pH may also greatly affect the mode of viral inactivation at different temperatures (Salo & Cliver, 1976).

6.2.4. Light

While wavelengths of ultraviolet light in sunlight are known for their ability to inactivate viruses by causing cross-linking among the nucleotides, earlier studies did not suggest that they played a significant role in virus inactivation (Kapuscinski & Mitchell, 1980; Wommack et al., 1996). However, more recent studies on phage indigenous (virioplankton) to the marine environment suggests that they could play a significant role even at depths as great as 200 m (Suttle & Chen, 1992; Wommack & Colwell, 2000). Even in turbid estuarine waters, a modest effect of sunlight on viral decay can occur at a depth of 2.5 m (Cottrell & Suttle, 1995). Light wavelengths of <320 (UV-B) have the greatest virucidal effect on virioplankton (Murray & Jackson, 1993). The effect of bacteriophage viability in seawater is directly proportional to the amount of sunlight (Garza & Suttle, 1998). A recent study demonstrated that several enteroviruses were inactivated more rapidly in seawater in the presence of sunlight than in the dark (Fujioka & Yoneyama, 2002). The rate of inactivation was much less in the winter than summer. The double stranded DNA viruses are significantly much more resistant to UV light inactivation than the enteroviruses, because they can use host cell repair enzymes to repair the UV light damage (Gerba et al., 2002).

Visible light penetrates much further in natural waters than UV light and it has been suggested that light in this spectrum may cause photodynamic inactivation of viruses. In

photodynamic inactivation, the virus is "sensitized" to photoxidation by interaction of the viral genome with certain substances (Wallis & Melnick, 1965). Numerous synthetic dyes and natural substances, such as ligins, fulvic acids, humic acids, and vitamins, may act as photosensitizers. Dyes adsorb radiation and selectively transfer that energy to dissolved oxygen, which is excited to its highly oxidized state. Environmental factors, such as pH, temperature, time, and dye concentration, largely control the sensitization of the virus by the photosensitizer. The length of light exposure and its intensity can also influence the rate of virus inactivation (Gerba et al., 1977b). The process is not inhibited by dissolved organics and readily takes place in seawater. Damage to the virus is cumulative. A photodynamic antiviral substance has been demonstrated in algae cells (Fukada et al., 1968), giving support to the idea that photochemical reactions may be important to viral inactivation in natural waters.

6.2.5. Heavy Metals

While heavy metals such as copper, silver, nickel, and zinc are known to have antiviral properties in solution, their concentrations in seawater are probably too low to have a significant effect on enteric viruses (Thurman & Gerba, 1989).

6.2.6. Salts

The survival of enteric viruses in natural seawater is almost always greater than freshwater, but this appears to be due more to the presence of antagonistic microorganisms than salts in solution (Kapuscinski & Mitchell, 1980). The concentration of salts may affect the thermal resistance of viruses (Wallis & Melnick, 1962).

6.2.7. Microflora

Laboratory studies have indicated that the microflora of natural waters have a significant influence on virus survival. The application of laboratory results to field conditions is difficult, since the microorganisms involved or metabolic products may exist in such low concentrations in nature as to have no significant effect on virus survival. For example, conducting survival experiments in water samples placed in containers in the laboratory can allow for the build-up of organic acids, resulting in a drop in pH which in itself will effect virus survival.

Treating natural seawater to eliminate or kill bacteria or fungi by processes such as, autoclaving, filtration, addition of antibiotics or treatment with ultraviolet light, increases the survival of viruses (Shuval et al., 1971; Kapuscinski & Mitchell, 1980; Fujioka et al., 1980). The greater antiviral activity of seawater compared to freshwater appears to be related to the presence of bacteria indigenous to seawater (Fujioka et al., 1980). A bacterium identified as *Vibrio marinus* was isolated which exhibited antiviral activity, but unfortunately this activity was lost while maintaining the organism under *in vitro* conditions (Gundersen et al., 1967). Another marine bacteria classified, belonging to the genus *Moraxella*, was isolated from Mediterranean Sea and was able to retain its antivirus activity after passage in the laboratory (Girones et al., 1989). The virus-inactivating agent could not be separated from the viable marine bacteria, indicating that the active agent(s) either remained associated with the organism, or had a very short lifetime, or both. The antiviral activity of the organism was highly specific for poliovirus. No antiviral activity was found against the other groups of enteroviruses, rotavirus SA-11, or phages of

Table 6.2. Possible biotic mechanisms of viral inactivation.

1. Enzymatic degradation
2. Adsorption
Prevention of virus adsorption to host cells or capsid uncoating
Denaturation of protein coat
Release of nucleic acid
Alteration of net electronegativity
3. Sensitization
Photodynamic
Enzymatic
4. Reducing agents
5. Oxidizing agents

enteric bacteria. A *Vibrio* and *Pseudomonas* were isolated from the Atlantic Ocean which displayed antiviral activity against several enteroviruses (Toranzo et al., 1982). Culture filtrates of these organisms gave virus inactivation rates comparable with those obtained with whole cultures suggesting that extracellular products are involved in the antiviral activity. Further studies suggested that viral inactivation takes place by deformation or denaturation of a critical site(s) in the capsid followed by enzymatic degradation (Toranzo et al., 1983).

There are probably many mechanisms by which the native biota influences viral survival. Several possibilities are listed in Table 6.2. It follows that viral inactivation and degradation proceed by no single event, but by a series or combination of events. This is probably especially true of most of the enteroviruses, which are unusually resistant to a wide variety of proteolytic enzymes (Cliver & Herrmann, 1972). The susceptibility of viruses to enzymatic attack may result after the virus has been altered or inactivated by nonbiological factors such as pH (Salo & Cliver, 1976). Thus, inactivation of a virus should not be considered synonymous with viral degradation.

There are conflicting reports as to the involvement of biological factors in virus survival, but it should be kept in mind that the microflora of natural waters are not uniform. Failure to demonstrate the involvement of bacteria in the inactivation of viruses in a given water sample may simply mean that an organism affecting virus survival was not collected in that particular sample. It has been observed that the relative importance of biotic factors varies from one water sample or body of water to another (LaBelle & Gerba, 1979; Herrmann et al., 1978).

Enteric viruses are usually considered more resistant to the action of proteolytic enzymes than many other groups of viruses (Chang, 1971), and this has been given as a reason for their long survival in natural waters. Polioviruses and Coxsackieviruses have been shown to be resistant to a wide range of proteolytic enzymes (Cliver & Hermann, 1972). However, this may vary with the serotype of enterovirus. *Pseudomonas aeruginosa*, which produces a proteolytic enzyme, significantly inactivated Coxsackievirus A9, and used the viral coat as a substrate. Studies in fresh waters have suggested that enteroviruses are degraded by the indigenous microflora (Cliver & Hermann, 1972).

Bacteria and other microorganisms may also produce substances, which inactivate viruses by processes other than enzymatic. *P. aeruginosa* has been found to produce substances with a molecular weight below 500, which appeared to result in the virus being dismantled. Substances with such a low molecular weight could act enzymatically. Bacteria also produce

substances that react with a virion to prevent its adsorption to host cells (Fujsaki et al., 1978). Microorganisms can also produce substances that do not directly inactivate viruses, but sensitize them to inactivation by other processes, i.e., photodynamic inactivation (Fukad et al., 1968) or enzymes (Salo & Cliver, 1978). Other products of microorganisms may act as oxidizing or reducing agents. This list includes such compounds as humic acids (Klocking & Sprossig, 1972), ascorbic acid (Salo & Cliver, 1978), phenolic compounds (Konowalchuk & Speirs, 1978), and tannin (Konowalchuk & Speirs, 1978).

6.2.8. Macroflora

Aquatic organisms produce substances that are detrimental to virus survival (Sigel et al., 1976). Substances may be produced which act to promote virus survival. For example, the survival of coliphage MS-2 was prolonged in the presence of a growing culture of algae (*Cylinrothecia closterium*) in seawater–sewage mixtures. Many marine animals such as sponges, the sea squirt algae, and fungi produce substances with antiviral properties (Donia & Hamann, 2003), however, it is not known if they play any role in enteric virus survival in marine waters.

6.2.9. Solid–Water Interface

Viruses are known to readily associate with particulate matter in water. Virus adsorption to solids appears to play a major role, not only in their hydrotransportation, but also in their survival in nature. Enteric viruses can be expected to be present in larger number in the sediment than the surface water of any sewage contaminated marine environment (Goyal et al., 1978; Rao et al., 1984). Adsorption may act to not only prolong virus survival, but such associations may also enhance virus inactivation (Gerba & Schaiberger, 1975; Murray & Laband, 1979). With regard to potential adsorbents found in natural waters, coliphages and human enteric viruses have been found to adsorb to sand, pure clays (e.g. montmorillonite, illite, kaolinite, and bentonite), bacterial cells, naturally occurring suspended solids, and estuarine silts and sediments. In addition, viruses may be discharged into natural waters already associated with solids (Gerba et al., 1978).

Adsorption of coliphages T7 and T2 to pure clays has been shown to greatly reduce inactivation rates in natural and artificial seawater (Gerba & Schaiberger, 1975; Bitton & Mitchell, 1974). Bacterial colloids and naturally occurring suspended matter in seawater also have a protective effect on the survival of coliphages. Estuarine sediments have been demonstrated to prolong the survival of human enteroviruses (De Flora et al., 1975; Smith et al., 1978). The more sewage pollution an area is receiving, the more protective effect the sediment appears to exhibit (Smith et al., 1978). Under field conditions sediment-associated viruses held in dialysis bags, results similar inactivation rates to laboratory studies (LaBelle & Gerba, 1979). The greater concentrations of enteric viruses found in estuarine sediments also suggest that under field conditions solid-associated viruses exhibit longer survival times (Gerba et al., 1977a).

Virus adsorption to container walls may also effect virus survival. Akin et al. (1976), in studying poliovirus type 1 survival in seawater, observed that the virus appeared to survive longer in those containers, which permitted adsorption to the walls. Poliovirus readily adsorbs to glass surfaces, but not to polycarbonate or polyethylene surfaces. Thus, the container in

which a laboratory viral survival experiment is conducted could conceivably influence the rate of viral decline.

The protective effect of clays on virus survival in seawater may be due to several factors, including the adsorption of enzymes or other substances that inactivate viruses, increased stability of the viral capsid, prevention of aggregate formation, and interference with action of virucidal substances. Clays such as kaolinite are capable of sorbing a great variety of inorganic and organic ions or other substances that could inactivate viruses (Gerba & Schaiberger, 1975). As an example, bentonite clays have been shown to protect viruses against inactivation by ribonuclease by the adsorption of this enzyme to the clay (Singer & Fraenkel-Conrat, 1961). Another explanation of the protective effect of clay could be the formation of an envelope of clay particles around the virus, which would prevent contact of the virus with virucidal agents (Roper & Marshall, 1978). Marine sediments and clays also protect viruses against thermal inactivation in seawater (Liew & Gerba, 1980).

Some types of particulate matter in natural waters may also be antagonoistic to viral survival (Gerba & Schaiberger, 1975). Surfaces such as SiO_2 have little effect on virus survival, but metal oxide surfaces such as CuO, substantially decrease viral infectivity during adsorption–elution (Murray & Laband, 1979).

6.2.10. Air–Water Interface

Bubbles in the sea surf can adsorb and carry viruses to the surface of the water to be propelled into the air (Baylor et al., 1977). This results in the concentration of viruses at the sea–air interface. Certain coliphages (MS2 and R17) have been shown to be rapidly inactivated at dynamic air—water–solid interfaces (Thompson & Yates, 1999). This effect was shown to increase as the ionic strength of solutions was increased. The more hydrophobic the viral capsid, the more pronounced is the effect.

Other Factors

Although viral aggregation has not been known to influence viral survival in natural waters, aggregates are known to exhibit an increased resistance to inactivation by used disinfectants (Sharp et al., 1975). Viruses within an aggregate can be protected from factors in the environment that would act to degrade or inactivate the virus. The degree of viral aggregation appears to depend not only on the type of virus, but also the specific strain (Floyd & Sharp, 1977; Totsuka et al., 1978).

Viruses may interact with substances that do not inactivate them, but mask their detection in tissue culture by blocking their adsorption to host cells. Substances present in grape juice were observed to be reactivated in the host animal or after exposure to digestive tract secretions or human blood serum (Anonymous, 1978). This effect is believed to be due to polyphenols, including tannin (Konowalchuck & Speirs, 1976, 1978), both of which occur in natural waters.

High hydrostatic pressures can inactive enteric viruses (Kingsley et al., 2002). A 7 $\log_{10}$ occurred when hepatitis A virus was exposed to 450 MPa for 5 min. However, poliovirus was unaffected by a 5 min exposure to 600 Mpa. Suspension of hepatitis A virus in seawater increased its resistance, suggesting a protective effect of the salts in seawater. Exposure of the virus to RNase indicated that inactivation was to do to stile alterations of viral capsid proteins.

Ammonia has been shown in several studies to cause the inactivation of enteric viruses and bacteriophages (Burge et al., 1983), with the nucleic acid apparently being the target of inactivation (Ward & Ashley, 1977).

CONCLUSIONS

Many factors can potentially play a role in the inactivation of human pathogenic viruses in marine waters, however, temperature, sunlight, and indigenous microflora appear to play the dominate role. The significance of each individual factor may vary from location to location and the time of year. For viroplankton it was estimated that solar radiation caused 1/4 to 2/3 of the viral decay and that high heat liable, high molecular-weight dissolved material (probably enzymes) appeared responsible for 1/5 of the decay (Noble & Fuhram, 1997). Most of our knowledge on human enteric viruses rests on studies conducted with enteroviruses and little is still known about the survival of other groups of enteric viruses in marine waters.

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