APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Apr. 1978, p. 685-689
0099-2240/78/0035-0685$02.00/0

Vol. 35, No. 4
Printed in U.S.A.

Role of Sediment in the Persistence of Enteroviruses in the Estuarine Environment

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Received for publication 14 November 1977

The survival of four enteroviruses commonly found in sewage effluents was examined when the viruses were adsorbed to marine sediments in estuarine water and compared with virus survival in estuarine water alone. Echovirus 1, coxsackieviruses B3 and A9, and poliovirus 1 survived longer when associated with marine sediment. When the estuarine water was polluted with secondarily treated sewage effluent, virus survived for prolonged periods in sediments, but not in the overlaying estuarine water.

The survival of viruses in marine and fresh waters has been examined recently by several laboratories (1, 3, 9, 17, 19). The primary factor involved in virus inactivation in these studies was temperature (3, 6, 15, 17, 19). Other factors believed to be involved in viral inactivation are salinity (1), solar radiation (3), bacterial antagonism (6, 21), chemicals (18), pollution (3, 17), suspended solids (9, 24), and the type of virus (6, 15).

Our laboratory recently developed a method which allowed us to detect animal viruses adsorbed to marine sediments in the vicinity of a sewage outfall releasing secondarily treated sewage into a coastal canal community waterway (8, 10). Higher concentrations of virus were found in the sediment than in the overlaying water. Gerba and Schaiberger (9) reported that clays added to seawater retarded inactivation of virus, whereas Schaub and Sagik (20) demonstrated that animal viruses adsorbed to clay retained their infectivity for mice and tissue culture.

The present study was designed to examine the survival of enteroviruses adsorbed to marine sediments. Untreated sediment and estuarine water were employed rather than pure clays and treated water, which have been used in previous investigations with bacteriophages (5, 9).

MATERIALS AND METHODS

Virus and viral assays. All viral assays were performed using the BGM cell line, which was passaged, grown, and maintained by previously described methods (16). The virus stocks were plaque-purified lines. The four viruses used were poliovirus 1 (strain LSc), echovirus 1 (strain Farouk) and coxsackieviruses B3 (strain Nancy) and A9 (strain Bozek). Stock virus was grown in BGM cells, concentrated 10-fold, partially purified by membrane chromatography (23), and stored at −30°C. Virus samples were diluted in tris(hydroxymethyl)aminomethane-buffered saline containing 2% fetal bovine serum, penicillin (100 U/ml), and streptomycin (100 μg/ml). Virus assays were performed by the plaque-forming unit method as used in this laboratory (22).

Study sites. The estuary water and sediments were collected from a coastal canal community bordering Galveston Bay, which is located along the upper Texas Gulf Coast. Two of the sites were receiving secondarily treated, unchlorinated domestic wastewater from sewage outfalls and from the drainage of septic tank effluents. The remaining site was a public bathing beach receiving no known wastewater discharge. Table 1 is a comparison of the physical characteristics of the material collected from the three sites. Sediment was collected with the aid of an Ekman dredge. Water samples were collected in sterile, flint-glass bottles at the surface.

Salinity. Salinity was determined by using an AO T/C refractometer (AO Instruments Corp., Buffalo, N.Y.).

UV light absorbance. The absorbance of ultraviolet (UV) light at a wavelength of 254 nm was measured in a Beckman model 24 spectrophotometer (Beckman Instruments, Inc., Fullerton, Calif.). The increased absorbance of UV light is relative to the soluble organic content of the water and can be used as an indicator of pollution (13).

Fecal coliform count. Fecal coliform determinations on field samples were done by the most probable number method according to *Standard Methods for the Examination of Water and Wastewater* (2). EC broth was used for the fecal coliform test.

Procedure for survival measurement. The survival experiments were initiated the same day the samples were collected. The basic setup for the experiment consisted of two systems. One system was a 250-ml flint-glass bottle (Brockway Glass Co., Inc., Brockway, Pa.) that contained 100 ml of estuarine water and the viral inoculum. The second system was an identical bottle containing 10 ml of wet sediment, 90 ml of overlaying estuarine water, and the viral inoculum. Each experiment consisted of 24 bottles representing the four virus strains, three sites, and two different

TABLE 1. *Site characteristics*

Sampling station	Nature of sediment	Surface water			
		Salinity (ppt)[a]	pH	Absorbance (254 nm)	No. of fecal coliforms[b]
1	Sand	29.0	8.3	0.043	<2
2	Mud, shell	25.0	8.3	0.133	24
3	Mud, sand	24.0	8.3	0.226	8,733

[a] ppt, Parts per trillion.
[b] Most probable number observed per 100 ml.

survival systems. The final concentration of the viruses was 1×10^5 PFU/ml in each bottle. Before sampling, the bottles were shaken by hand to mix thoroughly the sediment and virus. A sample was taken, diluted in tris(hydroxymethyl)aminomethane containing 2% fetal bovine serum, and frozen at −30°C until the samples could be assayed on the same lot of BGM cells. The technique of directly plating the sediment particles was used successfully by Gerba and Schaiberger (9) and has been shown to be an efficient indicator of the actual number of infectious units present (14). The virus detected in the assays where sediment particles were inoculated was probably eluted from the particles by the presence of fetal bovine serum in the diluent. Thus, viable virions are detected individually rather than as single infectious units.

RESULTS

The three sites varied considerably in the degree of pollution. Table 1 describes the physical parameters of the water and sediment for each site. The sediments all contained sand, but the shell and debris content varied. Previously published data (10) indicate that greater than 99% of the added virus is adsorbed to these different types of sediments.

The salinity of the different sites did not vary extensively. The values are typical for an estuarine environment. The pH was also normal for the area and was identical at all sites.

Table 1 demonstrates the relative degrees of pollution for the three sites. The least polluted site was no. 1, which had the lowest fecal coliform count and UV absorbance value. This site was located at a public bathing beach. Site 2, located in a coastal canal community, was exposed to a low amount of pollution from water runoff and septic tank drainage. Site 3 was the most polluted of the three areas, as shown by the high fecal coliform count and UV absorbance. Site 3 was located near a sewage outfall.

Figures 1 to 3 show the inactivation rates of the three virus strains in seawater alone and in seawater containing sediment. The ordinate of these figures is the $\log_{10} N_T/N_O$. N_T is the titer of the virus at a particular time point, and N_O is the initial titer of the virus. The abscissa is a time scale in days.

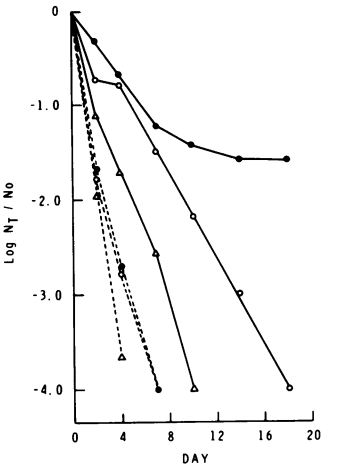

FIG. 1. *Survival of echovirus 1 in seawater and adsorbed to marine sediment in seawater. Symbols: (△---△) site 1 seawater; (△——△) site 1 seawater and sediment; (○---○) site 2 seawater; (○——○) site 2 seawater and sediment; (●---●) site 3 seawater; (●——●) site 3 seawater and sediment.*

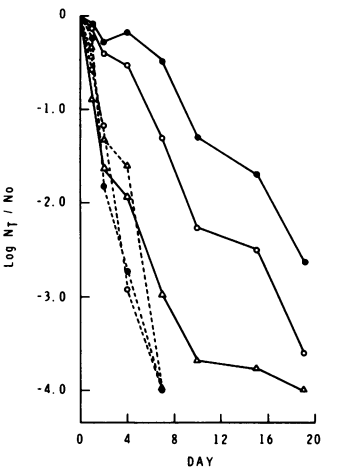

FIG. 2. *Survival of coxsackievirus B3 in seawater and adsorbed to marine sediment in seawater. See Fig. 1 for explanation of symbols.*

Echovirus 1 survived for the longest period of time in the sediment-containing system of site 3 (Fig. 1). Survival was significantly greater when adsorbed to sediments, even in the nonpolluted site 1. The rate of inactivation for echovirus 1 in seawater alone was the same and linear for all three sites. Since the sites differ in degree of pollution, this may be the cause for the prolonged survival of virus adsorbed to marine sediment.

Coxsackievirus B3 also survived longer in the systems containing sediment than in seawater

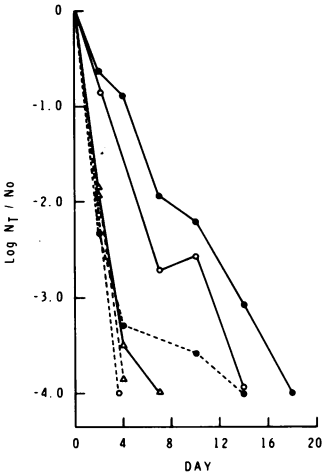

FIG. 3. *Survival of poliovirus 1 in seawater and adsorbed to marine sediment in seawater. See Fig. 1 for explanation of symbols.*

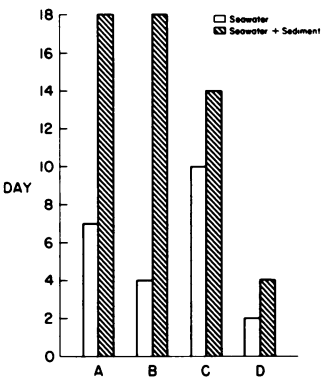

FIG. 4. *Comparison of the maximum length of survival of the four virus types in seawater alone and in seawater containing sediment. (A) Echovirus 1; (B) coxsackievirus B3; (C) poliovirus 1; (D) coxsackievirus A9.*

alone (Fig. 2). Survival was longest in the sediment from the most polluted site. Inactivation of coxsackievirus B3 and echovirus 1 were both very rapid in seawater alone.

Figure 3 shows the inactivation of poliovirus 1; essentially the same results as in Fig. 1 and 2 are shown. Survival was longest in the sample containing sediment from site 3. Samples containing seawater alone all had similar inactivation rates, and site 1, the least polluted site, had an initial inactivation rate similar to that of the seawater alone. This presumably was due to the lack of factors present in the sewage effluent.

Figures 1 to 3 demonstrate that for these enteroviruses survival was longer when adsorbed to marine sediment and was directly related to the pollution of the overlaying water.

Echovirus 1 and coxsackievirus B3 demonstrated the longest survival times (Fig. 4). The value for the maximum length of survival used in this figure was the last point in time at which infectious particles could be detected by plaque assay. The limit of detectability was a four-log reduction in N_T/N_O. Echovirus 1 and coxsackievirus B3 remained at detectable levels through the termination of sampling. The inactivation rate in the sample containing echovirus leveled off at a relatively high titer (Fig. 1). Inactivation in the sample containing coxsackievirus B3 continued at a high rate at the final time point (Fig. 2). The inactivation curves showed that echovirus 1 had the longest survival time, greater than 18 days, of the four enteroviruses tested; then followed coxsackievirus B3, 18 days; poliovirus 1, 14 days; and coxsackievirus A9, 4 days. In the samples containing seawater alone, coxsackievirus A9 was undetectable after 2 days.

DISCUSSION

The detection of animal viruses adsorbed to marine sediments indicates that they can serve as a reservoir of animal viruses (7, 8, 10). The high affinity of marine sediment for virus adsorption (10) and the detection of virus in sediments near sewage outfalls (7, 8, 10) suggest the importance of sediment in viral pollution. Sediment can be carried by water and distributed far from the original site of pollution (11), making sediment-borne virus hazardous and difficult to control. The present study examined the role of natural sediments in enterovirus survival in estuarine water. Naturally occurring sediment consists of many components that can have different effects on virus adsorption than single-component clays. Previous studies (5, 9) of virus survival when adsorbed to particulates used pure clays and/or treated water and were more removed from actual field conditions.

The major factors suggested to influence virus survival are temperature, salinity, pH, microbial population, and chemical agents (1, 12). The extent of the role these factors play in a sediment-containing system is unknown. Our study utilized estuarine water from the same site as the sediment. This equalized the exposure of the virus to water-mediated inactivating agents. The physical conditions such as temperature and light exposure were also identical for both systems. The waters from the three sites were similar except for their soluble organic content. Our laboratory demonstrated previously (10) that, under these conditions, greater than 99% of the virus is adsorbed to the marine sediment. The pH of the seawater is known to influence virus adsorption to solids, with greater adsorption of viruses at acid pH 4. Akin et al. (1) reported an

optimal salinity value of 10 g/kg in artificial seawater for virus adsorption.

It is only possible to speculate on the mechanism of virus protection by adsorption to sediment. The protective effect could be physical, such as trapping of the virus in a surface opening or stabilizing the virion by electrostatic forces. Sediment particles could also act as a buffer, adsorbing chemical inactivating agents present in the seawater (18). Shuval et al. (21) suggested that bacteria were involved in virus inactivation in seawater. Cliver and Herrmann (6) found variable responses for different virus strains to proteolytic enzymes. Our results (Fig. 1 to 4) show that sediment-adsorbed viruses have different rates of inactivation. Coxsackievirus A9 was inactivated in a shorter period of time than the other types of viruses. This strain of coxsackievirus was also the most susceptible to proteolytic enzymes in Cliver and Herrmann's study (6). Another difference in survival behavior among these three enteroviruses is shown in Fig. 1 to 3. Poliovirus 1 and coxsackievirus B3 were inactivated at the same rate for the first 3 to 4 days in the water and water-plus-sediment systems of site 1. Viral inactivation was not apparent until the titer had dropped to low levels. Conversely, echovirus 1 was protected by the site 1 sediment even at the high initial titer.

Site 3, the most polluted site, had the lowest inactivation rates of viruses adsorbed to sediment, perhaps because of a protective effect of viral adsorption to bacteria. Bitton and Mitchell (5) found that bacteriophage T7 was protected by the presence of *Escherichia coli* K. Since the bacteriophage does not infect this strain of *E. coli,* it presumably was protected from inactivation by adsorption to this biocolloid. Soluble organic matter present in the sewage effluent and interfering with chemical inactivating agents could be another mechanism enhancing virus survival under polluted conditions. Mitchell and Jannasch (18) noted that suspended organic matter enhanced ϕX174 survival.

The data on increased virus survival presented in this report, along with the reports of sediment-bound viruses detected in field studies (7, 8, 10), suggest that sediment-bound virus exists under natural conditions. Particulate-bound virus is infectious (20), so the sediment could serve as a vehicle for virus dissemination during sediment resuspension (11).

The enhancement of virus survival by pollution has been described in seawater (3, 17), but not with regard to marine sediments. This study demonstrated the protective effect adsorption to natural sediments has on the survival of enteroviruses in estuarine waters. Similar results were seen with bacteriophages T2 (9) and T7 (5) adsorbed to pure clays. This study and others (6, 15, 17) also have shown that different viruses are inactivated at different rates and that this is also true for sediment-associated enteroviruses. Finally, the presence of pollution was demonstrated to prolong the survival of viruses adsorbed to sediment. These results suggest that marine sediment may serve as a reservoir of human enteric viruses. Virus survival would benefit from pollution, and transport of virus-containing sediment by water circulation could represent a public health hazard that is not being monitored presently.

ACKNOWLEDGMENTS

This research was supported by the National Oceanic and Atmospheric Administration's Office of Sea Grants, Department of Commerce, through institutional grant 04-6-158-44108 to Texas A&M University.

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