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Effects of Bentonite Clay Solids on Poliovirus Concentration from Water by Microporous Filter Methods

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To determine whether suspended solids interfere with enteric virus recovery from water by microporous filter methods, the effects of bentonite clay solids at a concentration of 10 nephelometric turbidity units on the recovery of poliovirus type 1 from seeded, activated carbon-treated, filtered tap water were studied. Volumes (500 ml) of virus-laden water at pH 5.5 or 7.5, with and without 50 mM $MgCl_2$, were filtered through 47-mm-diameter, electropositive (Virosorb 1MDS) and electronegative (Filterite) filters that had been pretreated with Tween 80 to minimize direct virus adsorption to filter surfaces. Bentonite solids enhanced virus retention on both types of filters, even under conditions in which viruses were not solids associated. However, bentonite solids also interfered with elution of retained viruses when eluting with 0.3% beef extract–50 mM glycine (pH 9.5). Under some conditions, overall virus recoveries were lower from water with bentonite solids than from solids-free control water. The results of this study indicate that clay turbidity can interfere somewhat with virus recovery by current microporous filter methods.

Methods for concentrating enteric viruses from water by adsorption to and subsequent elution from microporous filters are now widely used to assess the virological quality of drinking water and other waters (1, 3, 5, 9, 12). The effectiveness of these methods can be limited by interferences from both dissolved and suspended matter naturally present in water (1). Although it is recognized that suspended solids can clog absorbent filters, thereby limiting sample volumes and possibly interfering with virus elution, there is little quantitative information on the effects of suspended solids on virus adsorption, elution, and overall recovery (15, 19, 20). The effects of suspended solids on microporous filter methods for virus concentration from water may be considerable because waterborne viruses are often largely solids associated (2, 4, 7, 8). The ability of microporous filter methods to recover solids-associated enteric viruses has not been adequately determined.

Recently, the effects of naturally occurring suspended solids in raw surface water and finished drinking water on microporous filter methods for enteric virus concentration were quantified (11). Although recovery efficiencies of seeded enteric viruses in raw and finished waters were not adversely affected by suspended solids, only one water source was examined, and the precise nature of the suspended solids was not determined. In this present study, suspended solids effects on poliovirus recovery from water by microporous filter methods were further examined in a model system consisting of activated carbon-treated, filtered tap water and bentonite clay, an inorganic suspended solid commonly present in natural waters.

MATERIALS AND METHODS

Virus and virus assays. Poliovirus type 1, strain LSc, was used as a model enterovirus. Viruses were grown and assayed as previously described (14).

Filters. Both electropositive and electronegative microporous adsorbent filters were used for virus concentration (10). The electropositive filter medium was Virosorb 1MDS (charge-modified fiberglass, 0.2-μm nominal porosity) (AMF Corp., CUNO Division). It was used in two layers as in filter cartridges.

The electronegative medium was Filterite (fiberglass-epoxy) (Filterite Inc.). Two layers of Filterite medium were used; the upper and lower layers were 0.45- and 0.25-μm porosity, respectively.

Disk filters (diameter, 47 mm) were placed in polypropylene housings (Millipore Corp.) and sterilized by autoclaving. To minimize direct virus adsorption to filter surfaces and thereby facilitate the determination of bentonite clay effects on virus retention and elution, filter assemblies were pretreated with 15 ml of a 1% Tween 80 (polyoxyethylene [20] sorbitan monooleate) solution for 15 min. After treatment, filter assemblies were rinsed with 300 ml of sterile, activated carbon-treated tap water to remove excess Tween 80. Tween 80 was used to pretreat filters because it is the most widely used, chemically defined agent known to minimize enterovirus adsorption to microporous filters (16–18).

To minimize the effects of soluble and colloidal organic matter on virus adsorption to either filters or bentonite clay, all experiments were done with dechlorinated (50 mg of sodium thiosulfate per liter) tap water from Chapel Hill, N.C., that was further treated by granular activated carbon (Filtrasorb 400; Calgon Corp.) filtration (adsorption), followed by filtration through a 0.2-μm-porosity polycarbonate filter (Nuclepore Corp.). Treated water had total organic carbon concentrations consistently less than 0.5 mg/liter (11).

Bentonite. Wyoming bentonite (American Colloid Co.), a montmorillonitic clay, was prepared by sedimentation techniques in sterile distilled water to remove soluble impurities and obtain particle sizes in the range of approximately 0.5 to 7.0 μm in diameter (6). Stock clay suspensions in sterile distilled water were added to treated water samples to give a

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TABLE 1. Effects of pH and $MgCl_2$ on poliovirus adsorption to bentonite clay in treated tap water

Water conditions		Initial poliovirus adsorbed (%) in:	
pH	$MgCl_2$ (mM)	Water only	Water with bentonite
7.5	None	<1	12
7.5	5	NT[a]	81
7.5	50	12	>99
5.5	5	NT	46
5.5	50	NT	>99

[a] NT, Not tested.

final turbidity of 10 nephelometric turbidity units (NTU), unless otherwise indicated.

Virus concentration experiments. Procedures for concentrating poliovirus from seeded water were similar to those previously described (10, 11). Treated tap water was seeded with poliovirus to give an initial concentration of about 10^4 PFU/ml. Bentonite was added to some samples to give a turbidity of 10 NTU. Samples were adjusted to desired pH levels and ionic conditions, and then 500-ml volumes were filtered at 50 ml/min through 47-mm-diameter adsorbent filters. Flow rates in samples containing bentonite often decreased due to filter clogging. Filtrates were collected and assayed for unadsorbed viruses. Viruses retained on filters were eluted with two 7.5-ml volumes of 0.3% beef extract in 50 mM glycine (pH 9.5) (13). Eluates were adjusted to pH 7.2 and assayed for recovered viruses.

RESULTS

Poliovirus adsorption to bentonite clay in treated tap water. As previously noted, enteric viruses in natural waters are often solids associated, and in this study poliovirus adsorption to bentonite in the model system could influence virus retention by microporous filters. Therefore, poliovirus adsorption to bentonite in treated water at pH and ionic conditions used for virus concentration was determined. In these experiments, 50-ml samples of treated water containing 10^4 PFU of poliovirus per ml were adjusted to pH 5.5 and 7.5, and some samples were amended with 5 or 50 mM $MgCl_2$, or 100 NTU of bentonite, or both. After mixing for 15 min at room temperature, samples were centrifuged at 10,000 × *g* for 20 min to sediment bentonite. The resulting supernatants were assayed for unadsorbed viruses. Poliovirus adsorption to bentonite was >99% in the presence of 50 mM $MgCl_2$ at both pH levels (Table 1). In the presence of 5 mM $MgCl_2$, poliovirus adsorption to bentonite was 81 and 46% at pH 7.5 and 5.5, respectively. There was little virus adsorption to bentonite in the absence of added $MgCl_2$. These results are generally consistent with those of Schaub and co-workers, who reported extensive enterovirus adsorption to bentonite in water amended with $CaCl_2$ (7, 8). In the absence of bentonite, there was little loss of poliovirus infectivity after centrifugation. The 12% adsorption from control tap water at pH 7.5 and containing 50 mM $MgCl_2$ could have been due to some virus adsorption to naturally occurring suspended solids or to the walls of the sample container. The results of these experiments demonstrate that under adsorption conditions typically used for Virosorb 1MDS and other electropositive filters (pH 7.5, no $MgCl_2$), poliovirus adsorption to bentonite was minimal, but under adsorption conditions typically used for Filterite and other electronegative filters (pH 5.5, with 5 or 50 mM $MgCl_2$), poliovirus adsorption to bentonite was extensive.

Poliovirus adsorption to Tween 80-treated filters. To determine whether pretreatment of filters with Tween 80 minimized direct virus adsorption to Virosorb 1MDS and Filterite filters, virus adsorption experiments were done with Tween 80-treated filters and treated water seeded with 10^4 PFU of poliovirus per ml. Water samples were adjusted to pH 3.5, 5.5, and 7.5 before filtration, and filtrates were assayed for unadsorbed viruses. Both types of filters were relatively poor virus adsorbents at pH 7.5 and 5.5, with considerable proportions of the initial poliovirus appearing in the filtrates (Table 2). The extent of poliovirus adsorption to Tween 80-treated Virosorb 1MDS filters at pH 5.5 and 7.5 was less than that previously obtained with untreated filters (10). At these same pH levels, Filterite adsorption of poliovirus is poor with or without Tween 80 treatment (10). At pH 3.5, however, poliovirus adsorption to both types of filters was extensive, with <1% of the initial viruses appearing in the filtrates. Absence of poliovirus in pH 3.5 filtrates was due to adsorption and not inactivation because most of the initial viruses (an average of 53%) were recovered by eluting the filters with 0.3% beef extract–50 mM glycine (pH 9.5). Due to extensive poliovirus adsorption to Tween 80-treated filters, subsequent experiments on poliovirus concentration from water containing bentonite were not done at pH 3.5.

Effects of bentonite on poliovirus concentration. Results of experiments on poliovirus concentration from treated tap water with and without 10 NTU of bentonite with both types of filters are summarized in Table 3 in terms of retention, elution, and overall recovery efficiencies, as percentages. Percent retention was determined from differences in virus concentrations between the initial, seeded water and the filtrates. Percent elution was determined from the amount of virus recovered in the eluate compared with the computed amount adsorbed to the filter. Percent recovery was determined from the amount of virus recovered in the eluate compared with the amount in the initial, seeded water. For Filterite samples without bentonite or $MgCl_2$, tabulated values for poliovirus recovery efficiencies are higher than would be computed from the values for adsorption and elution efficiencies. Thus, it appears that more viruses are recovered than would be predicted from the quantities of viruses adsorbed, as computed from the quantities detected in the filtrates. It is likely that such discrepancies arise because of generally poor virus adsorption under these conditions and some variability in the precision and accuracy of the virus assay method.

Under conditions typically used for Virosorb 1MDS (pH 5.5 or 7.5, no $MgCl_2$) and Filterite (pH 5.5 or 7.5, with $MgCl_2$), poliovirus retention efficiencies were generally greater in the presence of bentonite than in solids-free controls. Differences in virus retention efficiencies between samples with and without bentonite were statistically significant ($P < 0.05$) in two-sample *t*-tests. Previous experiments (Table 1) indicated that poliovirus was extensively (>99%)

TABLE 2. Effect of pH on poliovirus adsorption to filters treated with Tween 80

Adsorption pH	Initial viruses in filtrate (%) of:	
	Virosorb 1MDS	Filterite
7.5	47	80
5.5	36	54
3.5	0.6	0.08

TABLE 3. Effect of bentonite solids on poliovirus concentration from water with virosorb 1MDS and Filterite filters

Sample[a]	Filter type[b]	Adsorption conditions		Retention efficiency[c]	Elution efficiency[d]	Recovery efficiency[e]
		pH	$MgCl_2$ (mM)			
Control	Filt.	5.5	None	8.9 (18)[f]	2.0 (2.4)[f]	5.4 (4.2)[f]
	Filt.	5.5	50	88 (5.2)	71 (66)	62 (59)
	Filt.	7.5	None	20 (24)	5.9 (6.4)	4.2 (0.9)
	Filt.	7.5	50	85 (13)	43 (30)	36 (24)
Clay	Filt.	5.5	None	77 (24)	43 (22)	31 (14)
	Filt.	5.5	50	99 (1.7)	24 (13)	24 (13)
	Filt.	7.5	None	83 (18)	34 (28)	30 (20)
	Filt.	7.5	50	100 (0)	11 (7.0)	11 (6.9)
Control	1MDS	5.5	None	79 (22)	80 (28)	62 (23)
	1MDS	5.5	50	64 (12)	88 (52)	56 (29)
	1MDS	7.5	None	61 (7.0)	37 (8.5)	22 (4.6)
	1MDS	7.5	50	91 (9.0)	54 (35)	48 (26)
Clay	1MDS	5.5	None	99 (0.3)	32 (2.2)	32 (2.0)
	1MDS	5.5	50	100 (0)	50 (39)	50 (39)
	1MDS	7.5	None	96 (3.0)	24 (8.2)	23 (7.0)
	1MDS	7.5	50	93 (13)	13 (11)	12 (10)

[a] Control, Treated tap water; clay, treated tap water with bentonite clay at 10 NTU.

[b] Filt., Filterite; 1MDS, Virosorb 1MDS.

[c] Percentage of initial viruses in sample retained by filter at indicated conditions.

[d] Percentage of total adsorbed viruses eluted from filter.

[e] Percentage of initial viruses in sample recovered in eluate.

[f] Initial values are means from four trials; values in parentheses are standard deviations.

adsorbed in the presence of 50 mM $MgCl_2$ but not in the absence of $MgCl_2$. Therefore, improved retention of poliovirus by Filterite filters from waters containing 50 mM $MgCl_2$ and bentonite can be explained by virus adsorption to bentonite and extensive bentonite retention by the filters. However, improved poliovirus retention by Virosorb 1MDS filters in the presence of bentonite cannot be explained on this basis because poliovirus was not extensively adsorbed by bentonite in water with no added $MgCl_2$ (Table 1). Thus, in the case of Virosorb 1MDS filters, bentonite improved poliovirus retention even though the viruses were not solids associated. It is likely that the retention and accumulation of bentonite on Virosorb 1MDS filters resulted in increased poliovirus retention, perhaps by reducing the porosity of the filter medium, by decreasing flow rates and thus increasing virus contact times with adsorbent surfaces, or by providing additional virus adsorption sites.

For poliovirus retained by filters under typical adsorption conditions (pH 7.5 or 5.5 for Virosorb 1MDS and pH 5.5 or 7.5 with 50 mM $MgCl_2$ for Filterite), elution efficiencies were lower from samples containing bentonite than from corresponding solids-free controls (Table 3). Differences in elution efficiencies between samples with and without bentonite were statistically significant ($P < 0.05$ in two-sample *t* tests) for Virosorb 1MDS at pH 5.5 and 7.5 and for Filterite with 50 mM $MgCl_2$ at pH 5.5, but not at pH 7.5. These results indicate that under some adsorption conditions, the presence of bentonite on filter surfaces apparently interferes with virus elution when using 0.3% beef extract–50 mM glycine (pH 9.5) as eluent.

There was no consistent pattern in the net, overall effect of bentonite on poliovirus recovery efficiencies for all filter types and adsorption conditions tested. For Virosorb 1MDS, bentonite significantly reduced poliovirus recovery efficiencies ($P < 0.05$ in a two-sample *t* test) when adsorption was at pH 5.5, but it had no effect on recovery efficiencies when adsorption was at pH 7.5. For Filterite filters and adsorption conditions of pH 5.5 or 7.5 with 50 mM $MgCl_2$, overall poliovirus recovery efficiencies were lower in the presence of bentonite than in solids-free controls. However, the reductions were not statistically significant ($P < 0.05$ in two-sample *t* tests).

DISCUSSION

The results of this study demonstrate that bentonite clay at a turbidity of 10 NTU is capable of reducing poliovirus recoveries from water by some microporous filter methods, even if the viruses are not solids associated. Two opposing effects by bentonite were observed: (i) increased virus retention by microporous filters and (ii) decreased elution of retained viruses. The net, overall result of these two effects was either reduced virus recoveries or no difference in virus recoveries compared with solids-free controls. The reductions in virus recoveries in the presence of bentonite were not dramatic, and at least some portion of the initial viruses was recovered. These results are generally consistent with those of a previous study in which naturally occurring suspended solids in raw surface water and finished drinking water did not significantly reduce the recovery efficiencies of four enteric viruses, including poliovirus, even though they improved enterovirus retention by microporous filters under some conditions (11).

These findings have important implications for the detection of enteric viruses in waters containing suspended solids. Because viruses in most waters are often solids associated (2, 4, 7, 8), their recovery by microporous filter methods is likely to be influenced by this association. Even for viruses that are not solids associated, the presence of suspended solids in water is likely to influence their recovery by microporous filter methods.

In this study, viruses retained on filters in the presence of bentonite solids were eluted with reduced efficiency by 0.3% beef extract–50 mM glycine (pH 9.5), a widely used eluent. If elution efficiency was improved, however, it may be possible to improve overall virus recoveries by taking advantage of the increased virus retention on filters in the presence of suspended solids. Further studies are needed on the development of improved eluents and elution procedures for viruses retained on microporous filters in the presence of suspended solids.

Bentonite solids at a turbidity of 10 NTU did not dramatically interfere with overall recoveries of poliovirus by the various microporous filter methods tested. Therefore, it is likely that clay solids in water are a contributing, but perhaps not the most important, source of interference with virus recoveries by microporous filter methods. Indeed, dissolved and colloidal organic matter appear to be quantitatively more important sources of interference (11). However, further research is needed to determine the effects of other inorganic and organic suspended solids on enteric virus recoveries from water. Only until such additional studies are done will it be possible to understand the true potential and full extent of suspended solids interference with enteric virus recoveries from water by microporous filter methods.

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LITERATURE CITED

1. **American Public Health Association.** 1980. Standard methods for examination of water and wastewater, 15th ed., p. 848–871. American Public Health Association, Washington, D.C.
2. **Bitton, G.** 1975. Adsorption of viruses onto surfaces in soil and water. Water Res. **9**:473–484.
3. **Gerba, C. P.** 1983. Methods for recovering viruses from the water environment, p. 19–35. *In* G. Berg (ed.), Viral pollution of the environment. CRC Press, Boca Raton, Fla.
4. **Gerba, C. P., C. H. Stagg, and M. G. Abadie.** 1978. Characterization of sewage solid-associated viruses and behavior in natural waters. Water Res. **12**:805–812.
5. **Goyal, S. M., and C. P. Gerba.** 1982. Concentration of viruses from water by membrane filters, p. 59–116. *In* C. P. Gerba and S. M. Goyal (ed.), Methods in environmental virology. Marcel Dekker, New York.
6. **Jackson, M. L.** 1969. Soil chemical analysis—advanced course, 2nd ed. M. L. Jackson, Soil Science Department, University of Wisconsin, Madison.
7. **Schaub, S. A., and B. P. Sagik.** 1975. Association of enteroviruses with natural and artificially introduced colloidal solids in water and infectivity of solids-associated virions. Appl. Microbiol. **30**:212–222.
8. **Schaub, S. A., C. A. Sorber, and G. W. Taylor.** 1974. The association of enteric viruses with natural turbidity in the aquatic environment, p. 71–83. *In* J. F. Malina and B. P. Sagik (ed.), Virus survival in water and wastewater systems. University of Texas, Austin.
9. **Sobsey, M. D.** 1982. Quality of currently available methodology for monitoring viruses in the environment. Environ. Int. **7**:39–51.
10. **Sobsey, M. D., and J. S. Glass.** 1980. Poliovirus concentration from tap water with electropositive adsorbent filters. Appl. Environ. Microbiol. **40**:201–210.
11. **Sobsey, M. D., and J. S. Glass.** 1984. Influence of water quality on enteric virus concentration by microporous filter methods. Appl. Environ. Microbiol. **47**:956–960.
12. **Sobsey, M. D., J. S. Glass, R. J. Carrick, R. R. Jacobs, and W. A. Rutala.** 1980. Evaluation of the tentative standard method for enteric virus concentration from large volumes of tap water. J. Am. Water Works Assoc. **72**:292–299.
13. **Sobsey, M. D., J. S. Glass, R. R. Jacobs, and W. A. Rutala.** 1980. Modifications of the tentative standard method for improved virus recovery efficiency. J. Am. Water Works Assoc. **72**:350–355.
14. **Sobsey, M. D., H. R. Jensen, and R. J. Carrick.** 1978. Improved methods for detecting enteric viruses in oysters. Appl. Environ. Microbiol. **36**:121–128.
15. **Sobsey, M. D., C. Wallis, M. Henderson, and J. L. Melnick.** 1973. Concentration of enteroviruses from large volumes of water. Appl. Microbiol. **26**:529–534.
16. **Wallis, C., M. Henderson, and J. L. Melnick.** 1972. Enterovirus concentration of cellulose membranes. Appl. Microbiol. **23**:476–480.
17. **Wallis, C., A. Homma, and J. L. Melnick.** 1972. A portable virus concentrator for testing water in the field. Water Res. **6**:1249–1256.
18. **Wallis, C., A. Homma, and J. L. Melnick.** 1972. Apparatus for concentrating viruses from large volumes. J. Am. Water Works Assoc. **64**:189–196.
19. **Wellings, F. M., A. L. Lewis, and C. W. Mountain.** 1976. Viral concentration techniques for field sample analysis, p. 45–51. *In* L. B. Baldwin, J. M. Davidson, and J. F. Gerber (ed.), Virus aspects of applying municipal waste to land. University of Florida, Gainesville.
20. **Wellings, F. M., A. L. Lewis, and C. W. Mountain.** 1976. Demonstration of solids-associated virus in wastewater and sludge. Appl. Environ. Microbiol. **31**:354–358.