APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Jan. 1983, p. 323-327
0099-2240/83/010323-05$02.00/0

Vol. 45, No. 1

Simple Apparatus for Collecting Estuarine Sediments and Suspended Solids To Detect Solids-Associated Virus

THEODORE G. METCALF AND JOSEPH L. MELNICK*

Department of Virology and Epidemiology, Baylor College of Medicine, Houston, Texas 77030

Received 2 August 1982/Accepted 4 October 1982

Laboratory trials of a new sampler for collection of estuarine sediment-associated virus resulted in a recovery effectiveness averaging 30% for two enteroviruses and rotavirus SA11. A minimal recovery potential of 54% was calculated when losses caused by virus concentration procedure inadequacies were excluded. Both sediment-associated and suspended solids-associated viruses were collected with the sampler. Recoveries of 61 and 60% poliovirus and rotavirus, respectively, were obtained from salt water-suspended, solids-associated virus. The unique advantage of the sampler for selective collection of virus-associated top layers of sediment, plus collection over extensive areas, resulted in recovery of more virus than was obtained with a commonly used dredge-type sampler.

Virus adsorption to solids is a phenomenon of particular interest to environmental virologists (2). The solids-associated state in polluted aquatic environments has public health implications when human enteric viruses and recreational or shellfish-growing waters are involved (5, 12, 15). Marine sediments represent a potential reservoir of viruses contributing to the prolonged survival, transport, and uptake by shellfish of virus (4, 14). Widespread shellfish-transmitted illness illustrates the potential for health hazards that may result from creation of a shellfish-virus carriage status (8, 10, 11). Outbreaks of virus gastrointestinal illness among swimmers and bathers exposed to virus in recreational waters has invited further attention to the importance of solids-associated virus (SAV) to the survival and dissemination of these agents and to the possible transmission of virus illness by bathing in contaminated water (1, 3).

Collection of sediments for virus tests, as opposed to suspended solids, has been regarded as a different task, calling for use of different sampling equipment and techniques. Sediment samples usually have been collected with grab or dredge samplers which indiscriminately pick up sand, mud, and other sedimentary materials from which virus must be separated and recovered. Suspended solids are collected by passing water through filters from which retained solids and associated virus usually are recovered by backwash or elution.

A new sampler was developed principally for collection of sediments to be examined for virus, but was also capable of use for collection of suspended solids. The apparatus, called SAV sampler, represents a new approach to the collection of sediments to be examined for virus. Its development was based on the belief that SAV represents a spectrum extending from suspended to sedimented solids and that SAV may be found anywhere within this spectrum. A key element was the belief that virus adsorbs to small particulates that are probably colloidally or near colloidally dispersed and that these particulates settle out of a water column as floccules, forming a fluffy layer representing the uppermost portion of sediments. The more deeply located compact layers beneath probably contain little or no virus. The fluffy layers are easily disturbed, and it was believed that virus-associated solids would be picked up by the SAV sampler and deposited upon a collecting filter from which virus could be recovered by appropriate elution procedures.

The sampler illustrated in Fig. 1 was developed specifically for collection of the top fluffy sediment layer. It represents a modification of a Blue Devil Leaf Bagger used for collection of swimming pool sediments and debris. It is of plastic composition, 38 cm in diameter and 16.5 cm in height, and was obtained from Andrews Pool Company, Houston, Tex. Openings at the top of the enclosed bell-shaped structure allow water to pass through and permit unimpaired lowering of the sampler in a water column. Water is cycled by a gasoline-powered pump from sampler to cartridge-type filter and back to sampler. The sampler, pump, and filter are connected by high-pressure hosing outfitted with quick-connects. Water reentering the sampler at its base is forced through a ring with jet aper-

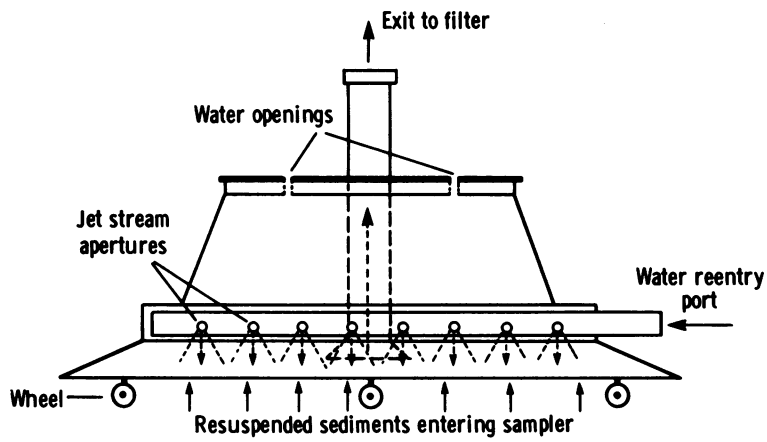

FIG. 1. SAV sampler.

tures opening downward. The uppermost fluffy layer, suspended by jet stream action directed against bottom sediments, is drawn up through the sampler and passed to a collecting filter where it is retained. Suspended solids collected from water columns are similarly passed to and deposited upon a collecting filter.

Laboratory tests of the new sampler were designed to determine how effective it was for recovery of SAV existing either as sediment-associated or suspended SAV. SAV was prepared by adsorption of virus to 2 g of kaolin and 8 g of silt loam (Wards Natural Science Establishment, Rochester, N.Y.) suspended in 400 ml of phosphate-buffered saline. Suspensions were mixed continuously for 45 min, followed by centrifugation and recovery of sediments containing adsorbed virus. SAV was determined as the difference between input and supernatant virus PFU. Test viruses included two enteroviruses, poliovirus 1 (LSc) and echovirus 1 (Farouk), and simian rotavirus SA11. The viruses were prepared in the form of monodisperse suspensions by freeze-thaw treatment of crude cell culture harvests, followed by passage of clarified suspensions adjusted to pH 8.5 through a series of Tween 80-treated membrane filters. Absence of significant differences in plaque assays of two consecutive filtrates was considered evidence of monodisperse virus. Assays were made on a 10-cm^2 monolayer surface with 0.1 ml of inocula adsorbed for 1.5 h at 37°C, followed by addition of overlay medium. Buffalo green monkey kidney monolayers were used with the enteroviruses (7), and fetal rhesus monkey kidney (MA104) monolayers were used with strain SA11 (13).

Tests were carried out in tanks of 567-liter capacity (bottom area, 7,254 cm^2) containing 75.7 liters of dechlorinated tap water (10 mg of sodium thiosulfate per liter). An estuarine salt environment was created by the addition of Instant Ocean (Aquarium Systems, Mentor, Ohio). Salinities were determined with a T/C refractometer (American Optical Corp., Buffalo, N.Y.), and turbidities were determined with a turbidimeter (Hach Portalab; Hach Chemical Co., Loveland, Colo.; model 16800). Fluffy layers of SAV on top of 2- to 3-cm-thick beds of sand were formed by the addition of kaolin-silt loam-adsorbed virus to tank water, followed by an 18- to 22-h settling interval. Samples with turbidities equivalent to those of Texas estuarine waters were filtered to determine volumes that could be tested before significant reduction of flow rate occurred. Samples with turbidities of 177 nephelometric turbidity units (NTU) could be passed through a filter in volumes greater than 230 liters before flow rates were affected. An average of 18.3 g of solids was recovered by backwash of filters.

The first set of experiments was designed to determine how much sediment-associated virus could be recovered from a simulated estuarine environment with the SAV sampler. Tank water (59 to 64 liters) was sampled. The fluffy layer picked up was deposited upon a Filterite 0.45-μm, 10-inch cartridge filter (Filterite Corp., Timonium, Md.) from which fluffy layer-associated virus was recovered by elution with 800 ml of 3% beef extract (pH 10.5). Fluffy layer solids recovered in eluates varied from 3.9 to 5.8 g. Eluates adjusted to pH 7.6 were concentrated to detect and determine virus PFU present. An organic flocculation method representing a modification of a previously reported method (6) was used to recover virus from filter eluates after adsorption to precipitated organic floccules. Recovered virus, suspended in a small volume of 0.05 M glycine, was frozen and finally, after thawing, clarified by centrifugation. Final sample volumes of 2.3 to 4.2 ml were obtained.

Results from control tests were used to follow the SAV status. Release of adsorbed virus and inactivation or loss of virus during tests was monitored.

The percentage of solids settling and forming a fluffy layer on sand beds, determined from 0- and 18- to 22-h tank water turbidity measurements, showed settling rates of ≈92%. Assays of 1-liter concentrates of tank water supernatants separated from solids showed that virtually all virus remained solids associated. Collection and assay of 1-liter concentrates of collecting filter filtrates failed to detect filter-passing virus. Tests of supernatants and sediments separated from 100 ml of tank water collected at 0 h, and after 18 to 22 h of holding at tank water temperatures, indicated the extent of virus losses during solids settling and the PFU remaining at the time sediment samples were collected. Losses averaging 11% occurred. Beef extract (3%), in 800-ml volumes, with a virus level identical to that used for preparation of virus-associated solids and concentrated the same way as test samples, showed the extent of virus losses incurred during concentration. Losses varied between 12 and 33%. Results from the first set of experiments are given in Table 1.

A minimum recovery potential of 54 and 47% for poliovirus and rotavirus, respectively, was calculated. This expression of relative effectiveness for recovery of sediment-associated virus represented PFU recovered in filter eluates. Calculated recovery potentials excluded losses incurred during concentration and represented the preconcentration percent recovery of sediment-associated virus remaining after an 18- to 22-h settling period. Recovery expressed as minimal recovery potential differentiated between the effectiveness of sediment collection and elution and the procedure for eluate concentration. Calculated recovery potentials were considered conservative. No correction was made for the loss of 8% sediment-associated virus estimated not to have settled on sand beds. Assumptions, based on visual observations, that all of the sediment had been collected may not have been completely correct. To the extent that less virus than assumed collected on the filters, recovery effectiveness would have been greater than calculated. Also, concentration losses averaging ≈22% could have masked the occurrence of filter-passing virus. Corrections for filter-passing virus would have increased recovery effectiveness. Actual recoveries of 32 and 25%, respectively, for poliovirus and rotavirus were the result of inadequacies of the concentration method used and were not indicative of the sampler recovery potential.

The next set of experiments sought to determine minimal recovery potentials for the SAV sampler and a conventional dredge-type sampler under simulated field sample collecting conditions unique to each sampler. An Ekman sampler (Wildlife Supply Co., Saginaw, Mich.) was used to collect samples at one point of the bottom sediment, simulating a field sample collection from bottom sediments at a given point. Collected samples of 37 to 68 g of sand and fluffy layer were eluted with 3% beef extract (pH 10.5), and the recovered virus was concentrated. SAV samples were obtained over most of the tank bottom area, simulating field sample collection over several square meters of bottom sediments. Samples were processed as described above for the first set of experiments. Results are given in Table 2.

Results of control tests were similar to those obtained in the first set of experiments. Greater minimal recovery potentials were obtained by the SAV sampler in each of the five trials, including two in which no recoveries were made with the Ekman sampler. An average of greater than 5 to 7 g of fluffy layer suspended solids in tank waters was collected. The greater SAV

TABLE 1. Recovery of fluffy layer-associated virus collected by the SAV sampler[a]

Trial	Virus	Virus input[b]	Cumulative virus loss (PFU)		Recoveries[c]		% Virus recovery	
			After 18–22 h	After concn	Solids	Virus	Filter eluate[d]	Eluate concn[e]
1	Poliovirus	239	40	141	4.2	61	62	31
2	Rotavirus	877	78	535	4.6	197	58	25
3	Rotavirus	622	80	292	3.9	123	37	23
4	Poliovirus	24	14	7	4.7	8	47	35

[a] Fluffy layer-associated virus resulting from 18 to 22 h of settling of kaolin- and silt loam-associated virus in 75.7 liters of salt water (sample volumes, 61 to 64 liters; salinity, 9 g/kg; turbidities: 0 h, 91 to 163 NTU; 18 to 22 h, 7 to 13 NTU). Virus PFU are given in hundreds.

[b] PFU of SAV added to salt water.

[c] Grams of solids (wet weight) recovered in eluate; PFU of virus recovered in filter eluate concentrates.

[d] Percentage of sediment-associated virus eluted from collecting filter.

[e] Percentage of virus recovered in final sample.

TABLE 2. Fluffy layer-associated virus minimal recovery potential obtained with SAV and dredge-type samplers[a]

Trial	Virus	Virus input[b]	Cumulative virus loss (PFU)		Minimum recovery potential[c]	
			After 18–22 h	After concn	Ekman sampler	SAV sampler
1	Poliovirus	273	39	167	2/106	52/106
2	Poliovirus	5,357	1,200	3,128	100/2,229	1,500/2,229
3	Echovirus	2,114	549	1,586	16/528	275/528
4	Rotavirus	10	1	4	0/6	3/6
5	Rotavirus	30	3	12	0/18	12/18

[a] Fluffy layer formed by 18 to 22 h of settling of kaolin- and silt loam-associated virus suspensions in 75.7 liters of salt water (sample volumes, 59 to 61 liters; salinity, 9 g/kg; turbidities: 0 h, 162 to 188 NTU; 18 to 22 h, 13 to 17 NTU). Virus PFU are given in hundreds.

[b] PFU of SAV added to salt water.

[c] PFU recovered/PFU remaining after losses incurred during settling and concentration procedures. Ekman samples, 37 to 68 g (wet weight); solids recovered in eluates of SAV-collected fluffy sediments, 4.7 to 5.8 g (wet weight).

recoveries were the result of selectivity of collection of fluffy layer solids, combined with more fluffy layer solids collected from most of the bottom area. This gave the SAV sampler unique advantages not possessed by the Ekman sampler.

The effectiveness of the SAV sampler for recovery of seawater-suspended SAV was determined in a third set of experiments. SAV was added to tank water as in previous experiments, but collection of suspended solids was made immediately, before the solids could settle upon bottom sand beds. Results of four trials are given in Table 3.

Control tests showed that 99% of the added solids were in suspension, and no dissociation of SAV was detected. No filter-passing virus was detected and, since concentration of collecting filter eluates was not necessary, no concentration losses were incurred. Recovery of 60% of the rotavirus input and an average recovery of 61% of the poliovirus showed that suspended solids-associated virus could be recovered effectively from filter-collected solids. Actual recoveries in the trials were very similar to the minimum recovery potentials calculated for the first set of experiments.

Results obtained in the three sets of experiments showed that the SAV sampler was a simple, versatile collector of either sediment-associated or suspended SAV. Virus recovery effectiveness was expressed as minimal recovery potential to better emphasize sampler collection capabilities. The effectiveness and ease of recovery was attributed to two factors: selectivity of collection of the solids most likely to contain adsorbed virus and a capability for collecting these solids over extensive bottom sediment areas. These factors contributed directly to a greater ease of separating and recovering virus from collected sediments, while simultaneously increasing the likelihood of virus recovery through collection of greater amounts of fluffy sediments. Possession of these advantages by the SAV sampler, but not the Ekman sampler, was the reason for the greater recoveries made with the former. Effectiveness of virus recovery depended upon the success of virus elution from collected fluffy sediments, regardless of whether they were filter or eluate associated. No appar-

TABLE 3. Recovery of SAV suspended in seawater[a]

Trial	Virus	Virus input[b]	Recovery[c] (eluate)		% Virus recovery
			Solids	Virus	
1	Poliovirus	46	5.3	35	76
2	Poliovirus	115	5.6	57	49
3	Poliovirus	95	5.1	56	59
4	Rotavirus	5	4.9	3	60

[a] Kaolin- and silt loam-associated virus suspension added to 75.7 liters of seawater (sample volumes, 62 to 66 liters; salinity, 19 g/kg; turbidities, 144 to 177 NTU.

[b] PFU of SAV added to salt water.

[c] Grams of solids (wet weight) recovered in eluate; PFU of virus recovered in eluate.

ent relationship between sediment position on a filter or in an eluate and elution effectiveness was found. Realization of minimal virus recovery capability was shown to depend upon avoidance of losses during concentration of eluates from collecting filters. Inadequacies of the method used for concentration reduced recovery effectiveness for sediment-associated virus to the vicinity of 30%.

Recovery effectiveness in the experiments was based upon suspended fluffy sediment turbidities measured in Texas estuarine water being sampled for virus. Turbidities in laboratory test volumes were equivalent to estuarine-suspended fluffy sediment turbidities measured in 193-liter sample volumes. The laboratory results were interpreted to indicate that SAV sampler-collected, 193-liter estuarine samples with suspended fluffy sediment turbidities $\leq$180 NTU and estimated filter-accumulated solids $\leq$15 g could be examined for sediment-associated virus without significant loss of recovery effectiveness. The extent of recovery effectiveness beyond this limit was not determined, but previous experiences with virus recovery from large volumes of turbid seawater indicate that eventually effectiveness is virtually certain to be adversely affected by increasing amounts of suspended sediment.

The SAV sampler represents a useful tool for studies of virus pollution of natural environments in which virus is associated with settled or suspended solids. It is not expected that the SAV sampler would be useful for recovery of freely suspended virus where pH adjustments, introduction of salt, and filter requirements call for different equipment and methods (9).

Virus recoveries obtained from estuarine sediments and suspended solids with the SAV sampler in Texas recreational and shellfish-growing waters, and the public health significance attached to these results, are described in a separate report.

This study was supported by the Sea Grant College Program, Texas A & M University, Office of Sea Grant, National Oceanic and Atmospheric Administration, U.S. Department of Commerce, and by a grant from the Environmental Protection Agency.

LITERATURE CITED

1. **Baron, R. C., F. D. Murphy, H. B. Greenberg, C. E. Davis, D. J. Bregman, G. W. Gary, J. M. Hughes, and L. B. Schonberger.** 1982. Norwalk gastrointestinal illness. An outbreak associated with swimming in a recreational lake and secondary person-to-person transmission. Am. J. Epidemiol. **115**:163–172.
2. **Bitton, G.** 1975. Adsorption of viruses onto surfaces in soil and water. Water Res. **9**:473–484.
3. **Bryan, J. A., J. D. Lehmann, I. F. Setiady, and M. H. Hatch.** 1974. An outbreak of hepatitis-A associated with recreational lake water. Am. J. Epidemiol. **99**:145–154.
4. **De Flora, S., G. P. De Renzi, and G. Badolati.** 1975. Detection of animal viruses in coastal seawater and sediments. Appl. Microbiol. **30**:472–475.
5. **Gerba, C. P., and S. M. Goyal.** 1978. Detection and occurrence of enteric viruses in shellfish: a review. J. Food Prot. **41**:743–754.
6. **Gerba, C. P., E. M. Smith, and J. L. Melnick.** 1977. Development of a quantitative method for detecting enteroviruses in estuarine sediments. Appl. Environ. Microbiol. **34**:158–163.
7. **Melnick, J. L., H. A. Wenner, and C. A. Phillips.** 1979. Enteroviruses, p. 471–534. *In* E. H. Lennette and N. J. Schmidt (ed.), Diagnostic procedures for viral, rickettsial and chlamydial infections, 5th ed. American Public Health Association, Washington, D.C.
8. **Murphy, A. M., G. S. Grohmann, P. J. Christopher, W. A. Lopez, G. R. Davey, and R. H. Millsom.** 1979. An Australia-wide outbreak of gastroenteritis from oysters caused by Norwalk virus. Med. J. Aust. **2**:329–333.
9. **Payment, P., C. P. Gerba, C. Wallis, and J. L. Melnick.** 1976. Methods for concentrating viruses from large volumes of estuarine water on pleated membranes. Water Res. **10**:893–896.
10. **Portnoy, B. L., P. A. Mackowiak, C. T. Caraway, J. A. Walker, T. W. McKinley, and C. A. Klein, Jr.** 1975. Oyster-associated hepatitis. Failure of shellfish certification programs to prevent outbreaks. J. Am. Med. Assoc. **233**:1065–1068.
11. **Roos, B.** 1956. Hepatitis epidemic conveyed by oysters. Sven. Laekartidn. **53**:989–1003.
12. **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.
13. **Smith, E. M., M. K. Estes, D. Y. Graham, and C. P. Gerba.** 1979. A plaque assay for the simian rotavirus SA11. J. Gen. Virol. **43**:513–519.
14. **Smith, E. M., C. P. Gerba, and J. L. Melnick.** 1978. Role of sediment in the persistence of enteroviruses in the estuarine environment. Appl. Environ. Microbiol. **35**:685–689.
15. **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.