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Poliovirus-1 Inactivation and Interaction with Biofilm: a Pilot-Scale Study

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A pilot-scale study was initiated to examine the behavior of viruses pulse injected into a distribution system. The influence of a free-chlorine residual and that of virus preadsorption to clay particles was evaluated by tracing the viruses both in the water flow and after elution from the biofilm. These experiments demonstrated, first, that virus preadsorption on 40 mg of Na-montmorillonite per liter increased the residence time of the viruses within the pilot plant by roughly three times and, second, that preadsorption to clay did not prevent viruses from being inactivated by chlorine. Moreover, with no clay added, a greater amount of viruses was recovered from the biofilm than from the water flow (by a factor of 2 or 10 in the absence or presence of chlorine, respectively), indicating a tendency for virus accumulation within biofilms.

Biofilm development within water distribution systems is an important source of concern, for it is known to impair drinking water quality. So far, most of the studies related to biofilms have focused on their bacteriological aspects (5, 19, 24, 25, 29, 32, 41, 42, 44), and those works involving virus detection in drinking water did not report virus elution from biofilms (14, 18, 33). The only study concerning the detection of enteric viruses in biofilms, as far as we know, was that of vanden Bossche and Krietemeyer (39), who reported the detection of various strains of type B coxsackieviruses within the biofilm colonizing the outlet mains of a drinking water treatment plant.

This observation may shed new light on epidemiological data which reveal that viruses are an etiological agent responsible for more than one-third of the waterborne-diseases outbreaks reported in North America (9, 16, 26) or in Europe (1, 10). Also, the contribution of the water distribution network to the morbidity related to the consumption of distributed water is well established (28).

Thus, if viruses can indeed accumulate within biofilms, it should be possible to trace them after their injection, both in the water flow and after elution from the biofilm. A pilot-plant study was initiated, because it was the only safe way to trace detectable amounts of viruses some distance away from the inoculation point. This study aimed in particular at demonstrating the role of viral inactivation and that of viral adsorption to the biofilm. Since viruses in cases of contamination probably enter the distribution network in association with particulate matter (23, 27, 31, 35, 38), the influence of virus adsorption onto clay particles was investigated in the presence and absence of a chlorine residual.

During preliminary studies and for analytical purposes, the network pilot plant was hydrodynamically characterized by using a saline tracer. A series of six experiments was then conducted. These involved pulse inoculations of known amounts of viruses into the pilot plant and recovery of the viruses after various intervals both in the water flow and after desorption from biofilm patches. Viral responses obtained under various conditions (i.e., in the presence or absence of a chlorine residual, with or without preadsorption onto clay particles) were then analyzed by using a data-fitting model and the concept of residence time distribution (34) applied to virus particles.

MATERIALS AND METHODS

Virus preparation and quantitation. A suspension of purified poliovirus 1 (Sabin LSc 2ab) obtained as described previously (30) was used throughout the experiments. Each sample (or a dilution thereof) was assessed for the presence of viruses by microscopic examination of cytopathic effects. For this purpose, BGM kidney cells seeded in 96-well microtiter plates were used, as detailed in reference 30. The virus titer was expressed as the most probable number of cytopathic units (MPNCU) per milliliter, using three dilution levels of 40 wells each, as explained previously (21). Each determination was performed in duplicate, including the whole procedure from sampling to titration. The confidence interval associated with each virus titer benefited from a substantial reduction (48 to 32% from mean titer) when the determinations did not differ from one another at the 5% level, according to the test of maximum likelihood (22).

Clay preparations and use. SW1-type Na-montmorillonite originating from Crook County, Wyo., was further purified to remove impurities and to render it homoionic to sodium (11). Preadsorption of viruses to clay was performed in the following manner. A sterile clay suspension was inoculated with a virus suspension and magnetically stirred for 30 min at 4°C. Then a $CaCl_2$ solution (final concentration, 10^{-2} M) was added and the whole suspension was stirred for an additional 30 min at 4°C.

Water. The viral suspensions inoculated into the pilot plant were prepared with milli-Q (Millipore) water. The pilot plant was continuously fed throughout the experiments with drinking water treated with the following steps. After microsieving, Seine River water was first coagulated and flocculated with $AlCl_3$, sand filtered, and bank percolated. Then the filtered water was further nitrified, ozonized, GAC filtered, and postchlorinated before being stored and distributed. The drinking water obtained had an average turbidity of 0.1 nephelometric turbidity unit, a conductivity of 0.6 mS/cm, and a total organic carbon content close to 1 mg/liter. After incubation for 7 days at 22°C, heterotrophic plate counts on R2A agar medium averaged 57 CFU/ml ($n = 23$).

Pilot plant. An open system was fabricated with a 90-m-long high-density polyethylene loop having a 26.5-mm internal diameter and a 50-liter capacity. A pump allowed for a 500-liter/h flow rate within the loop. The pilot plant also benefited from a double feeding system which allowed a thiosulfate feeding (0.03 liter/h) and an inlet water flow (5 liters/h) which was also used for the virus pulse injections (with a three-way valve). Flow rates were checked a few hours before each injection experiment. At the entry point of this double feeding system, the pilot plant was equipped with a static in-line mixer to provide rapid and thorough mixing. Sample withdrawal from the water phase occurred at the pilot plant outlet. Biofilm samples were collected from 1-cm^2 polyvinyl chloride (PVC) patches which had previously been thoroughly washed, sterilized, and fitted on the inside wall of the pipe 1 month before each experiment (Fig. 1).

Bacterial elution and quantitation. In order to estimate the number of bacteria colonizing the surfaces of PVC patches installed on the pipe for about 1 month, the following procedure was adopted. The patches were fitted onto specific sterile adapters, and 15 ml of filter-sterilized saline was added. After cooling on a salty melting-ice cushion, the film material was subjected to an intermittent (0.5 s on, 0.5 s off) sonication for 2 min with a 2.9-mm-diameter

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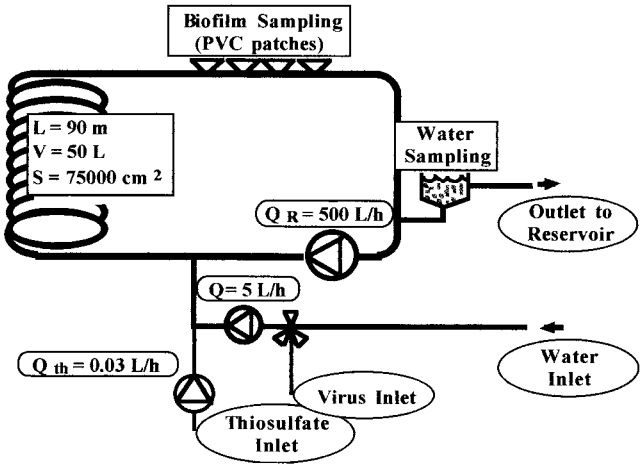

FIG. 1. Scheme of the pilot plant network at CIRSEE (not to scale). L, length of loop; V, capacity of loop; S, surface area; Q, water inflow rate; Q_R, recirculation flow rate; Q_{th}, thiosulfate inflow rate.

sonication probe placed 1 cm from the surface of the patch. The power indicator of the sonicator (Labsonic U2000; B. Braun Scientific) displayed a value of 40. Temperature increase in the adapter did not exceed 5°C under these conditions. After sonication, the resulting bacterial suspension was serially diluted and a DAPI (4′,6-diamino-phenyl-2-indole) solution was added to each bacterial dilution at the final concentration of 2.5 μg/liter. After 15 min, the stained suspensions were filtered under a vacuum through sterile 0.22-μm-pore-size polycarbonate membranes. The membranes were then examined with a UV light microscope, and the fluorescing DAPI-stained cells were counted on 10 fields of the membrane surface for each determination.

Chlorine level control and chlorine residual measurements. Continuous sodium thiosulfate feeding of the pilot plant was set up a few hours before the beginning of each experiment. Thiosulfate concentrations were calculated to partially or totally inactivate the free-chlorine residual within the pilot plant system. The free-chlorine residual was measured at the beginning of each experiment by the colorimetric *N,N*-diethyl-*p*-phenylenediamine–ferrous ammonium sulfate (DPD-FAS) technique, as described previously (12). The detection limit of the technique appeared to be below 0.04 mg/liter.

Pulse injections. For each experiment a freshly prepared 0.5-liter viral suspension was injected into the pilot plant system for 6 min. The first four experiments (a to d) were conducted with virus only, whereas the last two (e and f) involved a clay-virus-salt suspension (the initial virus load ranged from 3.5×10^3 to 9.0×10^3 MPNCU/ml). Experiments a to d were conducted with continuously injected $Na_2S_2O_3$ solutions calculated to reach average concentrations in the pilot plant system of 0, 1.1, 12.5, and 200 mg/liter, respectively. Experiment e required 200 mg of thiosulfate/liter as well, leading to an undetectable level of free chlorine, and none was added for experiment f.

Virus elution procedure. At given intervals after the pulse injection, PVC patches (biofilm samples) were withdrawn from the pilot plant and their colonized surfaces were immediately placed into contact with 1 ml of a 0.1 M Tris buffer (pH 8) and chilled on ice. After being shipped to the laboratory in a frozen state, the biofilm-colonized patches were fitted on specific sterile adapters and subjected first to a 5-min vortex (Heidolph Instruments; position 4 of 9) and then to a 30-min thorough mixing (on a shaking table at ca. 250 rpm) in the presence of sterile glass beads (diameter, 2 mm) and a glycine buffer (pH 9). As suggested by vanden Bossche and Krietemeyer (39), Tween 80 at a final concentration of 0.04% was added to the buffer because of a dissolved organic carbon content in the drinking water of 1 mg/liter. Elution samples were then withdrawn and subjected to a decontamination step using the following mixture at a 10% final concentration: 10 g of streptomycin/liter, 15 g of neomycin/liter, 10^8 IU of penicillin G/liter, and 50 mg of amphotericin B/liter.

Percentage of virus recovery and virus mean retention time. Based on the analysis of the residence time distribution of molecules introduced into the pilot plant (34), the percentage of virus recovery from the water compartment (%Rec) could be estimated as follows:

$$\%\mathrm{Rec} = 100 \cdot \int_0^\infty \frac{Q \cdot C(t)}{n_0}\,dt = 100 \cdot \frac{1}{\tau} \cdot \int_0^\infty \frac{C(t)}{C_0}\,dt$$

where Q, $C(t)$, and n_0 represent flow rate, concentration at time t, and initial amount of tracer, respectively. Similarly an estimation of the mean retention time ($\bar{t}$) was obtained by numerical integration as follows:

$$\bar{t} = \int_0^\infty tC(t)dt \Big/ \int_0^\infty C(t)dt$$

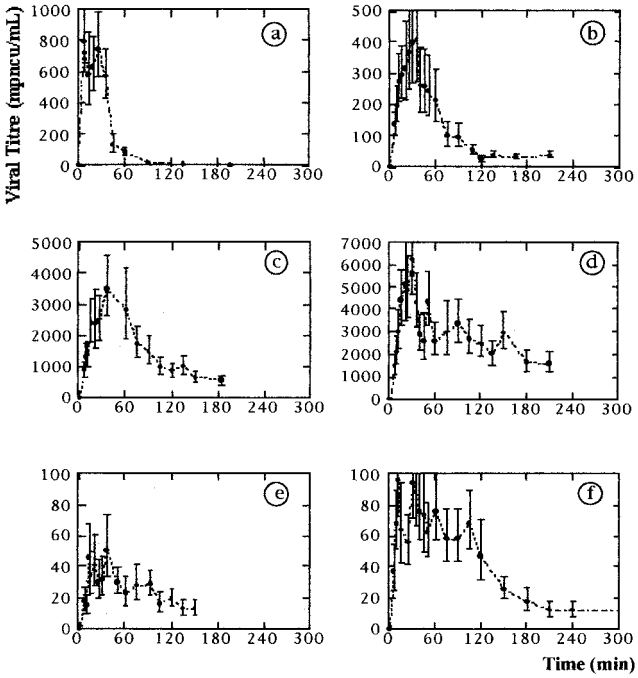

FIG. 2. Viral responses (mean titers of duplicates) in the water compartment at the pilot plant outlet. Poliovirus 1 initial concentrations in the pilot plant water were 9.0×10^3, 3.9×10^3, 9.0×10^3, 4.1×10^3, 3.5×10^3, and 3.5×10^3 MPNCU/ml for experiments a through e, respectively. Chlorine was present in experiments a through c and f. Clay was present in experiments e and f.

RESULTS

Virus responses in the bulk water. The six pulse injection experiments were carried out on a pilot plant system fed with very slightly chlorinated water: 0.04 mg of Cl_2/liter was measured at the pilot plant inlet, and a lower content could hardly be detected at the pilot plant outlet. Density of the biofilm grown in 1 month on the patches that were fitted on the pipe at the pilot plant averaged 10^6 cells/cm^2.

Viral responses in the water phase at the pilot plant outlet during the first 5 h after injection are reported in Fig. 2. For each determination the mean value of the duplicates (from sampling to titration) was considered. Gamma functions (i.e., in the form $f(t) = a \cdot t^b \cdot e^{-c \cdot t}$, where t stands for the time variable and a, b, and c are numerical parameters) successfully fitted all the responses (r^2 ranged between 0.92 and 0.98). For Fig. 3 the mean virus titers were normalized by the corresponding virus load introduced into the pilot plant, thus allowing comparison between the recorded answers, and only the modeling curves were considered for clarity.

Estimations of %Rec and of $\bar{t}$ were obtained by numerical integration according to the modeling plots. These calculations are reported in Table 1.

Percent recovery of viruses introduced into the pilot plant ranged from 0.6% in the presence of 0.02 to 0.04 mg of free chlorine/liter to 27.8% in the absence of chlorine (experiment d). Similarly, viruses remained in the water compartment four

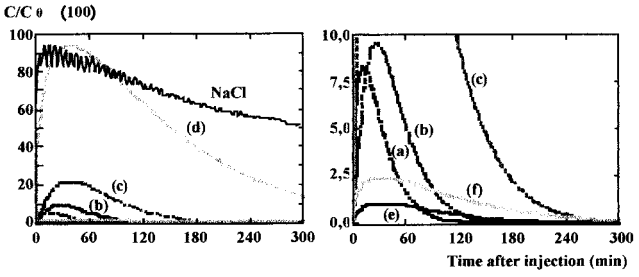

FIG. 3. Compact display of modeled viral responses (gamma function) of Fig. 2. The right-hand diagram is a magnification of the left-hand diagram. Letters indicate individual experiments.

times longer in the absence of chlorine than in the presence of a chlorine residual (120 versus 30 min) (Table 1).

When viruses were adsorbed on clay particles before introduction into the pilot plant, the overall percentages of recovered viruses were equally low both in the presence (experiment f) and absence (experiment e) of a chlorine residual (0.2 and 0.6%, respectively). In the same manner, in the presence of clay, viruses displayed estimated mean retention times in the water compartment of 105 and 91 min in the presence (experiment f) and absence (experiment e) of chlorine, respectively. Conversely, comparison between experiment d (no chlorine, no clay) and experiment e (no chlorine, 40 mg of montmorillonite/liter) revealed that in the absence of chlorine, adsorption of viruses onto clay particles dramatically changed the virus' behavior in the pilot plant system. For instance, after adsorption on clay (experiment e), 100 times fewer viruses were detected at the pilot plant outlet 30 min after injection than in the case of experiment d for equivalent virus loads (4.1×10^3 and 3.5×10^3 MPNCU/ml for experiments d and e, respectively). Nevertheless, in the presence of clay (experiment e) the overall virus retention time within the pilot plant was not very much shorter than that observed in the absence of clay (experiment d (91 versus 120 min) (Table 1). Comparison between experiments a and f, both conducted with a free-chlorine residual but in the absence or presence of clay, respectively, did not reveal any difference in the percentages of retrieved viruses, which in both cases was very low (0.6%) due to the high decay rate. Conversely, in the presence of clay (experiment f), virus mean retention time in the water compartment averaged four times that observed in its absence (experiment a). Moreover, 24 h after injection virus detection was still positive (1 MPNCU/ml) for experiment f, whereas no virus could be detected after about 2 h in the absence of clay (experiment a).

TABLE 1. Percentages of recovered viruses and residence mean times of viruses in the water compartment[a]

Expt	Clay[b]	Concn of chlorine[c]	r^2	%Rec	$\bar{t}$ (min)
a	−	+++	0.95	0.6	30
b	−	++	0.98	0.9	46
c	−	+	0.98	5.2	69
d	−	−	0.92	27.8	120
e	+	−	0.94	0.2	91
f	+	+++	0.96	0.6	105

[a] Estimated after fitting of a gamma function to the experimental data.
[b] +, present; −, absent.
[c] Free-chlorine concentration ranged between 0 (−) and 0.04 (+++) mg/liter.

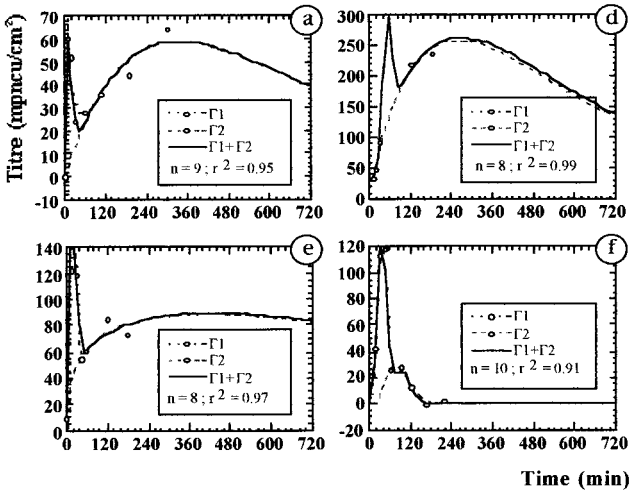

FIG. 4. Viral responses (mean titers of duplicates) in the biofilm compartment after elution and modeling with gamma functions. Data from experiments a and d through f are shown.

Virus responses in the biofilm. In all six experiments some viruses were actually recovered from the biofilm. Viral responses after elution from the biofilm-covered PVC patches are shown in Fig. 4. Although available patches for each experiment were limited in quantity, we successfully fitted the experimental data with the sum of two gamma functions (r^2 ranged between 0.91 and 0.99). The first one described a peak of biofilm contamination which coincided with the one observed for the water flow. This peak was followed by the other part of the viral response, unfortunately more weakly characterized but still able to be described by a second gamma function.

Interestingly, viruses could be retrieved from the biofilm about 1 day after injection, in both the presence (experiment a) and absence (experiment d) of a chlorine residual, but none was detected 4 h after injection in the presence of both clay and chlorine (experiment f) (Table 2).

In the case of experiment d (no chlorine, no clay), the percentage of retrieved viruses in the water compartment was estimated to be ca. 30%. Under the hypothesis that there was no virus inactivation, roughly 70% of the introduced viruses should have thus been recovered from the biofilm. Based on this hypothesis, the elution efficiency of the procedure used to recover viruses from the biofilm patches was estimated to be 18%, and %Rec for the other experiments was accordingly calculated (Table 2).

TABLE 2. Influence of clay and chlorine on virus recovery from biofilm[a]

Expt	Clay[b]	Chlorine[b]	Time (h) after injection	Virus concn (MPNCU/cm²) (CI)[c]	%Rec		
					From water	From biofilm[d]	Total
a	−	+	16	24 (18–32)	0.6	8.9	9.5
d	−	−	24	24 (18–32)	27.8	72.2	100.0
e	+	−	28	52 (39–68)	0.2	81.1	81.3
f	+	+	4	0	0.6	2.2	2.8

[a] Demonstrated by the duration of positive detection in the biofilm and by the percentage of virus recovery from the biofilm.
[b] +, present; −, absent.
[c] CI, confidence interval.
[d] Calculation based on the hypothesis of a maximum virus recovery for experiment d (%Rec [total] = 100%).

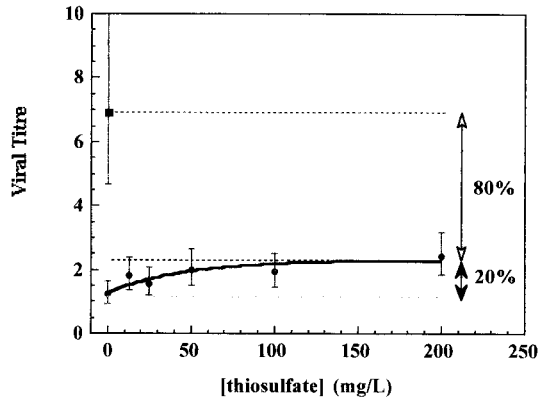

FIG. 5. Poliovirus 1 inactivation in the pilot plant feeding water after storage in the dark for 5 h at 25°C in the presence (●) and absence (■) of thiosulfate. Viral titers are expressed as 10^4 MPNCU/ml.

DISCUSSION

Viral responses in water: influence of chlorine. It is remarkable that such a slight free-chlorine concentration, i.e., at the detection threshold of the DPD-FAS technique, was enough to inactivate more than 1 log unit of poliovirus 1 in 30 min, even though the virus was a laboratory strain (20, 36). Some authors (7, 17) have shown that inactivation of enteric viruses by chlorine is not a linear function of the disinfectant dose. For instance, vanden Bossche et al. (40) observed a relationship between the poliovirus 1 inactivation rate and the residual ozone dose which followed a logistic model. This kind of relationship would explain how such a small change in chlorine concentration could lead to an important increase in the ability to inactivate viruses.

Additionally, a possibility of synergy between chlorine and other factors exists, as first demonstrated by Berg et al. (3, 4), who observed a 10-fold-faster poliovirus 1 inactivation by chlorine in drinking water than in milli-Q water.

As a matter of fact, when the equation $C/C_0 = f(t)$ (Fig. 3) is used for both the saline tracer experiment and Experiment d (no chlorine), the maximum of these responses, i.e., where t is approximately 40 min, almost reaches the value of 1. This means that for experiment d, the inactivation rate, k, can be considered null (C/C_0 amplitude is not lowered). Nonetheless, in comparison to the NaCl experiment, $\bar{t}$ for viruses in experiment d was about 4.2 times lower ($\bar{t}_{NaCl}$ = 589 min). Thus, if the effect of virucidal substances other than chlorine cannot be detected within short periods (≤40 min), the divergence between NaCl response and experiment d response which occurs for longer periods after injection could be due in part to other virucidal agents contained in the pilot plant feeding water. Indeed, an additional experiment carried out in closed bottles filled with the pilot plant feeding water supplemented with various amounts of thiosulfate indicated that 80% of the observed poliovirus 1 inactivation was attributable not to chlorine but to other dissolved (<0.22 μm in diameter) substances. The pilot plant feeding water actually displayed a great virus inactivation ability, as the T_{90} (time for a one-log-unit inactivation) for poliovirus 1 has been estimated to be 2.7 days (confidence interval, 2.4 to 3.0), considering a decreasing exponential model. Additionally, samples of the pilot plant feeding water were collected in closed sterile bottles and stored in the dark for 5 h at 25°C. From 0 to 200 mg of thiosulfate/liter was then added to the different bottles. As shown in Fig. 5, maximal viral inactivation (100%) after 5 h at 25°C was determined by comparison of the virus titers obtained in the absence of thiosulfate with and without storage. We observed that the chlorine residual in the pilot plant feeding water was responsible for only one-fifth of the overall viral inactivation. Similarly, the T_{90} value obtained in the unfiltered feeding water was 10 times smaller than that formerly observed at 25°C in sterile deionized water (11). Other studies have reported similar observations, and some demonstrated a virus-inactivating capacity of biological origin (6, 8). Actually, inactivation is not the only phenomenon that could lead to the divergence over time between the experiment d and NaCl responses. The observed difference could also correspond to a virus transfer from the water compartment to the biofilm compartment. This hypothesis is indeed worthy of consideration, as we were able to retrieve viruses from the biofilm.

Viral responses in water: influence of clay. Comparison of the number of viruses recovered from the water flow in the presence and absence of clay leads us to speculate that virus preadsorption to clay induces an important virus transfer from the moving phase to the stationary phase. This finding is consistent with a recent report (43) indicating a higher bacterial biofilm accumulation rate in the presence of clay particles. It also appears that the presence of 40 mg of montmorillonite/liter allows an increase in the period during which viruses can be detected in the water compartment, regardless of the presence of a chlorine residual. In particular, a protective effect of clay against chlorine for viruses is not observed, as the overall amount of retrieved virus is similar in the presence (experiment f) and absence (experiment a) of clay. Thus, comparison of the mean retention times and of the duration of positive detection periods (Table 1) leads us to the conclusion that the impact of chlorine on viruses would simply be delayed in the presence of clay, as other authors previously observed for poliovirus 1 and coliphage MS-2 (13, 37). Other nonclay particles such as organic matter may provide associated viruses with an effective protection against disinfection (2, 13, 15). In comparison to clay material, organic matter could thus bring about an increased virus survival in the water flow if viruses are completely embedded within the organic matrix. Conversely, since organic matter should be much more easily biodegraded than clay, at the biofilm level the virus residence time, as well as the impact of a system contamination, could be shortened.

Viral responses in biofilm. Here again, the percentages of virus retrieval from the biofilm reveal the virus sensitivity to the chlorine residual. According to the experiments conducted in the absence of chlorine (experiments d and e), the hypothesis of a virus transfer to the biofilm seems to be acceptable because of the high rates of virus recovery from the biofilm whether viruses were adsorbed to clay particles or not. Actually, virus preadsorption could favor this transfer, as viruses in experiment e appeared to be mainly distributed within the biofilm.

The low rates of virus recovery from both compartments observed for experiment f seem to indicate that for the concentration tested, clay particles do not provide viruses with any protective effect against chlorine. However, since the proportion of viruses that effectively reached the biofilm and that were not already inactivated can be questioned, the possibility of virus protection by the biofilm still cannot be excluded.

The results of this study indicate that when viruses introduced into the pilot plant system were not inactivated, they were distributed in both the bulk-phase water and the biofilm but were concentrated in the biofilm 2- to 10-fold more than in the bulk phase. Our results also demonstrate that virus adsorption to clay favors a virus transfer to the biofilm, inducing an increase of the virus retention time within a given length of the

system. Moreover, the presence in a distribution system of fine (1 or 2 μm in diameter) particulate matter does not seem to provide adsorbed viruses with protection against chlorine.

Use of a pilot plant system is the only simple alternative for tracing viruses both in the water flow and in the biofilm after their massive introduction into the system. Characterization of the pilot plant is a prerequisite for analysis of the residence time distribution of viruses within the pilot plant system. This analysis tool allowed us to observe the following: virus adsorption onto 40 mg of montmorillonite/liter increased roughly threefold the virus residence time within the system but did not protect viruses from chlorine inactivation, and in every case, a greater number of viruses was recovered from the biofilm than from the water flow. With no clay added, 2 and 10 times more viruses were recovered from the biofilm in the absence and presence of chlorine, respectively.

Along with viral inactivation, viral adsorption to biofilm is thus a leading determinant of the fate of viruses introduced into a distribution system and, in particular, would allow their accumulation within the biofilm, as first reported in 1995 (39). Further experiments are needed to investigate the control of these phenomena that allow for a viral accumulation within biofilms.

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REFERENCES

1. **Andersson, Y., and T. A. Stenström.** 1986. Waterborne outbreaks in Sweden—causes and etiology. Water Sci. Technol. **18:**185–190.
2. **Baldry, M. G. C., M. S. French, and D. Slater.** 1991. The activity of peracetic acid on sewage indicator bacteria and viruses. Water Sci. Technol. **24:**353–357.
3. **Berg, G., H. Sanjaghsaz, and S. Wangwongwatana.** 1990. KCl potentiation of the virucidal effectiveness of free chlorine at pH 9.0. Appl. Environ. Microbiol. **56:**1571–1575.
4. **Berg, G., H. Sanjaghsaz, and S. Wangwongwatana.** 1989. Potentiation of the virucidal effectiveness of free chlorine by substances in drinking water. Appl. Environ. Microbiol. **55:**390–393.
5. **Bryers, J. D., and W. G. Characklis.** 1982. Processes governing primary biofilm formation. Biotechnol. Bioeng. **24:**2451–2476.
6. **Carlson, G. E., Jr., F. E. Woodward, D. F. Wentworth, and O. J. Sproul.** 1968. Virus inactivation on clay particles in natural waters. J. Water Pollut. Control Fed. **40:**R80–R106.
7. **Churn, C. C., G. D. Boardman, and R. C. Bates.** 1984. The inactivation kinetics of H-1 parvovirus by chlorine. Water Res. **18:**195–203.
8. **Cliver, D. O., and J. E. Herrmann.** 1972. Proteolytic and microbial inactivation of enteroviruses. Water Res. **6:**797–805.
9. **Craun, G. F.** 1993. An overview of statistics on acute and chronic water contamination problems, p. 5–15. *In* Fourth Domestic Water Quality Symposium: point-of-use treatment and its implications. Water Quality Association, Lisle, Ill.
10. **Cubitt, W. D.** 1991. A review of the epidemiology and diagnosis of waterborne viral infections. Water Sci. Technol. **80:**40–52.
11. **Gantzer, C., F. Quignon, and L. Schwartzbrod.** 1994. Poliovirus-1 adsorption onto and desorption from montmorillonite in seawater. Survival of the adsorbed virus. Environ. Technol. **15:**271–278.
12. **Greenberg, A. E., R. R. Trussell, and L. S. Clesceri (ed.).** 1989. Standard methods for the examination of water and wastewater, 17th ed. American Public Health Association, Washington, D.C.
13. **Hejkal, T. W., F. M. Wellings, P. A. LaRock, and A. L. Lewis.** 1979. Survival of poliovirus within organic solids during chlorination. Appl. Environ. Microbiol. **38:**114–118.
14. **Hejkal, T. W., B. Keswick, R. La Belle, C. P. Gerba, Y. Sanchez, G. Dreesman, B. Hafkin, and J. L. Melnick.** 1982. Viruses in a community water supply associated with an outbreak of gastroenteritis and infectious hepatitis. J. Am. Water Works Assoc. **74:**318–321.
15. **Hoff, J. C.** 1978. The relationship of turbidity to disinfection of potable water, p. 103–117. *In* C. W. Hendricks (ed.), The evaluation of the microbiology standards for drinking water. EPA-570/9-78-00 C. U.S. Environmental Protection Agency, Washington, D.C.
16. **Hunter, P. R.** 1994. Drinking-water associated disease outbreaks. Microbiol. Eur. **2:**8–12.
17. **Jensen, H., K. Thomas, and D. G. Sharp.** 1980. Inactivation of coxsackieviruses B3 and B5 in water by chlorine. Appl. Environ. Microbiol. **40:**633–640.
18. **Keswick, B. H., C. P. Gerba, H. L. DuPont, and J. B. Rose.** 1984. Detection of enteric viruses in treated drinking water. Appl. Environ. Microbiol. **47:** 1290–1294.
19. **Lazarova, V., and J. Manem.** 1995. Biofilm characterization and activity analysis in water and wastewater treatment. Water Res. **29:**2227–2245.
20. **Liu, O. C., H. R. Serachekas, E. W. Akin, D. A. Brashear, E. L. Katz, and J. J. Hill.** 1971. Relative resistance of twenty human enteric viruses to free chlorine in Potomac water, p. 171–197. *In* V. Snoeynik (ed.), Virus and water quality: occurrence and control. Proceedings of the 13th Water Quality Conference. University of Illinois, Urbana, Ill.
21. **Maul, A.** 1991. Aspects statistiques des méthodes de quantification en virologie, p. 143–171. *In* L. Schwartzbrod (ed.), Virologie des milieux hydriques. Technique et Documentation Lavoisier, Paris, France.
22. **Maul, A., J. C. Block, and A. H. El-Shaarawi.** 1985. Statistical approach for comparison between methods of bacterial enumeration in drinking water. J. Microbiol. Methods **4:**67–77.
23. **Moore, R. S., D. H. Taylor, L. S. Sturman, M. M. Reddy, and G. W. Fuhs.** 1981. Poliovirus adsorption by 34 minerals and soils. Appl. Environ. Microbiol. **42:**963–975.
24. **Ohashi, A., D. G. Viraj de Silva, B. Mobarry, J. A. Manem, D. A. Stahl, and B. E. Rittmann.** 1995. Influence of substrate C/N ratio on the structure of multi-species biofilms consisting of nitrifiers and heterotrophs. Water Sci. Technol. **32:**75–84.
25. **Olson, B. H., R. McCleary, and J. Beeker.** 1991. Background and models for bacterial biofilm formation and function in water distribution systems, p. 225–285. *In* C. J. Hurst (ed.), Modelling the fate of microorganisms in the environment. VHC, Stuttgart, Germany.
26. **Payment, P.** 1993. Viruses: prevalence of disease, levels, and sources, p. 99–113. *In* G. F. Craun (ed.), Safety of water disinfection: balancing chemical and microbial risks. International Life Sciences Institute Press, Washington, D.C.
27. **Payment, P., E. Morin, and M. Trudel.** 1987. Coliphages and enteric viruses in the particulate phase of river water. Can. J. Microbiol. **34:**907–910.
28. **Payment, P., L. Richardson, J. Siemiaticki, R. Dewar, M. Edwardes, and E. Franco.** 1991. A randomized trial to evaluate the risk of gastrointestinal disease due to consumption of drinking water meeting current microbiological standards. Am. J. Public Health **81:**703–708.
29. **Pedersen, K.** 1990. Biofilm development on stainless steel and PVC surfaces in drinking water. Water Res. **24:**239–243.
30. **Quignon, F., and L. Schwartzbrod.** 1995. Influence of salts and montmorillonite upon heat inactivation of poliovirus in sterile water. Water Sci. Technol. **31:**177–180.
31. **Rao, V. C., K. M. Seidel, S. M. Goyal, T. G. Metcalf, and J. L. Melnick.** 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.
32. **Rijnaarts, H. H. M., W. Norde, E. J. Bouwer, J. Lyklema, and A. J. B. Zehnder.** 1993. Bacterial adhesion under static and dynamic conditions. Appl. Environ. Microbiol. **59:**3255–3265.
33. **Rose, J. B., and C. P. Gerba.** 1986. A review of viruses in drinking water. Curr. Pract. Environ. Eng. **2:**119–143.
34. **Sardin, M., D. Schweich, F. L. Leij, and M. T. van Genuchten.** 1991. Modeling the nonequilibrium transport of linearly interacting solutes in porous media: a review. Water Resourc. Res. **27:**2287–2307.
35. **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. Environ. Microbiol. **30:**212–222.
36. **Shaffer, P. T. B., T. G. Metcalf, and O. J. Sproul.** 1980. Chlorine resistance of poliovirus isolants recovered from drinking water. Appl. Environ. Microbiol. **40:**1115–1121.
37. **Stagg, C. H., C. Wallis, and C. H. Ward.** 1977. Inactivation of clay-associated bacteriophage MS-2 by chlorine. Appl. Environ. Microbiol. **33:**385–391.
38. **Stotzky, G.** 1980. Surface interactions between clay minerals and microbes, viruses and soluble organics, and the probable importance of these interactions to the ecology of microbes in soil, p. 230–247. *In* R. C. W. Berkeley, J. M. Lynch, J. Melling, R. P. Rutter, and B. Vincent (ed.), Microbial adhesion to surfaces. Ellis Horwood, Chichester, United Kingdom.
39. **vanden Bossche, G., and S. Krietemeyer.** 1995. Detergent conditioning of biofilm samples: the most sensitive method for the detection of enterovirus infectivity. Presented at the IAWQ 17th Biennial Conference, Budapest, Hungary, July 1994.
40. **vanden Bossche, G., U. Wustmann, and S. Krietemeyer.** 1994. Ozone disinfection dynamics of enteric viruses provide evidence that infectious titer reduction is triggered by alterations to viral colloidal properties. Microbiol. Res. **179:**1–20.
41. **van Loosdrecht, M. C. M., J. Lyklema, W. Norde, and A. J. B. Zehnder.** 1989. Bacterial adhesion: a physicochemical approach. Microb. Ecol. **17:**1–15.
42. **van Loosdrecht, M. C. M., D. Eikelboom, A. Gjaltema, A. Mulder, L. Tijhuis, and J. J. Heijnen.** 1995. Biofilm structures. Water Sci. Technol. **32:**35–43.
43. **Vieira, M. J., and L. F. Melo.** 1995. Effect of clay particles on the behaviour of biofilms formed by Pseudomonas fluorescens. Water Sci. Technol. **32:**45–52.
44. **Zhang, T. C., and P. L. Bishop.** 1994. Density, porosity, and pore structure of biofilms. Water Res. **28:**2267–2277.