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NOTES

Seasonal Occurrence of Rotavirus in Sewage

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A seasonal distribution was observed for rotaviruses in sewage by using indirect immunofluorescence. Levels were low from May through September and generally higher during winter and spring. In contrast, no seasonal pattern was observed for total enteroviruses. Limitations of the indirect immunofluorescence assay and enzyme immunoassay for environmental samples are discussed.

Rotaviruses are now recognized as a major cause of severe infantile diarrhea (2, 7, 10, 19). Rotaviruses may also cause gastroenteritis in adults either by contact with infected infants (8, 22), through contaminated water (21), or other mechanisms (1, 16).

The distribution of rotaviruses in water has not been studied before because of the lack of a suitable method for detecting rotaviruses at relatively low concentrations. Methods for detecting rotaviruses in environmental samples have only recently been developed. Steinmann (20) reported detection of rotavirus in concentrates of 2-liter sewage samples, using both an enzyme-linked immunosorbent assay and electron microscopy. Smith and Gerba (18) developed an indirect immunofluorescence (IIF) assay for detection of rotaviruses in water and used it to quantify levels of rotaviruses in raw and treated sewage. Although these studies have demonstrated that rotaviruses may occur in sewage at relatively high levels, nothing is known about the frequency with which it occurs or the seasonal fluctuations in rotavirus levels.

Many infectious diseases exhibit seasonal variations, and there is some evidence that infantile diarrhea from rotaviruses may be most prevalent in the fall and winter months (7, 14). If this is true, then levels of rotaviruses in sewage should increase during these months.

The present study was conducted to determine the seasonal distribution of rotaviruses and to compare it with the seasonal distribution of enteroviruses. Two methods for detecting rotaviruses, IIF and a commercial enzyme immunoassay (EIA), were compared for efficacy in quantifying rotaviruses from environmental samples.

Samples (20 liters) of untreated sewage entering a municipal sewage treatment plant in Houston, Tex., were taken at 2-week intervals from January 1979 through December 1980. Additional samples were taken at weekly intervals during the first 3 months of the study to determine short-term variability. Samples were collected between 9 and 11 a.m. to minimize the effects of diurnal variations.

A microporous filter adsorption-elution procedure was used to concentrate the samples as previously described (18).

During year 2 of the study, sample concentrates that were negative for rotaviruses in preliminary tests were concentrated further by hydroextraction overnight at 4°C in dialysis bags covered with polyethylene glycol flakes (24). The concentrated sample was clarified by centrifugation at 8,500 × *g* for 30 min and filtered through a positively charged depth filter (Zeta-plus, type 50S; diameter, 25 mm; AMF-CUNO, Meriden, Conn.) to reduce cytotoxicity (4).

Rotavirus levels in the concentrated samples were measured by the IIF test described previously (18). Positive controls with simian rotavirus SA11 of known titer were run with each assay. Also, for each sample, a negative control was run in which normal serum was added instead of anti-SA11 serum during the staining procedure.

Enteroviruses in each sample concentrate were quantified by a plaque assay on BGM (Buffalo green monkey) cells as previously described (3, 13). All plaques observed on BGM cells are reported as enteroviral PFU. No attempt was made to identify the numerous enterovirus isolates.

The concentrations of rotaviruses in Houston sewage over the 2-year study period are plotted in Fig. 1. No correction has been made in the reported values for efficiency of concentration. The most noticeable feature of the distribution is the occurrence of large fluctuations over short (≤1-week) time periods. Levels of rotaviruses in sewage ranged from 1 to 321 fluorescent foci (FF) per liter. The geometric mean of rotavirus levels over the entire 2-year period was 9.8 FF per liter. Levels below the detection limit were included in the calculation of the geometric mean by assigning them values of one-half the detection limit. Major peaks in rotavirus concentrations occurred during the spring of year 1 (March and April) and in the months of November and December of both years. Periods of consistently low levels occurred from May through September of both years 1 and 2. The detection limit was lowered from 5 FF per liter to 1 FF per liter by reconcentration of negative samples in the last half of year 2.

Concentrations of enteroviruses also exhibited extreme variability (Fig. 2). Enterovirus levels ranged from 7.5 to 800 PFU/liter. The geometric mean of enterovirus concentrations for the 2-year period was 70 PFU liter. No periods of sustained low or high levels were evident with the enteroviruses. Concentrations of enteroviruses at or above the geometric mean value were observed for all months in at least one of the two study years except for the months of August, September, and October.

Patchy distribution in space and time is characteristic for

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waterborne enteric viruses. This distribution can be attributed to a combination of factors including aggregation, association with solids, periodic large doses introduced into the sewage system, and changes in flow and water quality (5, 6, 23). These factors will contribute to high-frequency (hourly to daily) fluctuations in virus levels compared with the fluctuations expected from substantial changes in the total amount of virus being excreted by the population or from seasonal effects (e.g., temperature) on virus survival. The wide variations in rotavirus and enterovirus levels found in our study were similar to variations reported for other enteric viruses in sewage (6).

Seasonal variations in the levels of the individual groups of enteric viruses in sewage have been reported, and these variations roughly coincide with variations in the incidence of disease and infection from these viruses (9, 11, 12). We found that rotaviruses had a marked seasonal distribution, with higher concentrations occurring in late fall through early spring and lower concentrations occurring in summer. This distribution corresponds with reported frequencies of rotaviral diarrhea (7, 14).

No substantial dependence on seasonal factors could be discerned in the distribution of total enteroviruses. This may be because these data include a wide range of serologically distinct viruses, so that although individual types or groups may vary seasonally, no seasonal variation was detected in the composite of enteroviruses. An additional factor that would reduce seasonal variation is the use of live poliovirus vaccine in the population under study. These explanations are supported by the work of Irving and Smith (6), who demonstrated that many serotypes could be isolated only in certain months but that polioviruses and untyped enteroviruses were isolated in every month of their year-long study of treated sewage in Australia.

Based on tests with a human fecal specimen, each FF in the IIF test may represent at least 3.8×10^5 rotavirus particles. Thus, observed concentrations of 100 FF per liter indicate that over 10^7 rotavirus particles are present in each liter of sewage. The number of particles necessary to initiate infection or to cause disease is unknown. In the case of related reoviruses, the physical particle-to-PFU ratio is about 10 to 1 for virus grown in tissue culture (17). Because each FF represents such a large number of virions, detection of even a single fluorescing cell becomes important. Results of these assays should be interpreted by an experienced observer. Only cells showing an unquestionable characteristic fluorescence should be scored as positive. A negative control must be run for each sample and a positive control for each assay that is performed.

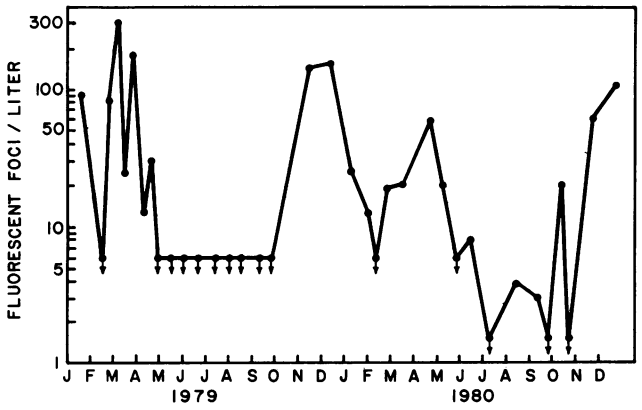

FIG. 1. Seasonal distribution of rotavirus in Houston sewage. Arrows indicate below detection limit. Negative samples after May 1980 were reconcentrated, resulting in a lower detection limit.

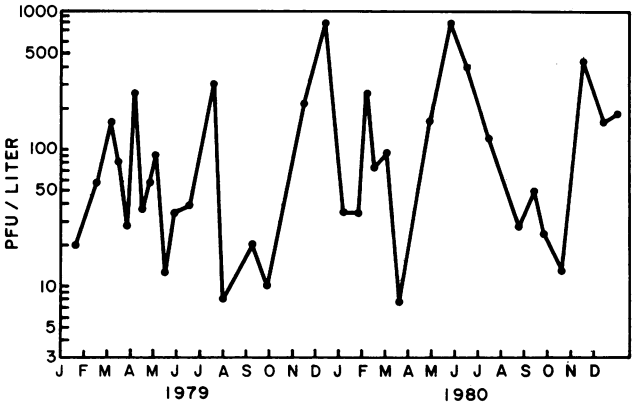

FIG. 2. Seasonal distribution of enteroviruses in Houston sewage. (Note change in scale from Fig. 1).

Because cytotoxicity was a problem with highly concentrated samples in the IIF test, 75 sewage concentrates were also tested by a commercially available EIA for rotavirus (Rotazyme; Abbott Laboratories, North Chicago, Ill.). Values obtained by EIA did not correlate well with the IIF values for these samples, and when samples that were positive in initial EIA tests were reassayed to perform blocking tests (15), 17 of 75 (23%) were found to be false-positives.

Thus, although EIA may prove useful for rapid tests, especially of cytotoxic samples, it seems essential that future studies with EIA for measuring rotavirus levels in water incorporate stringent controls such as simultaneous blocking assays to be sure that positive tests are due to the presence of rotaviruses.

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LITERATURE CITED

1. **Bolivar, R., R. H. Conklin, J. J. Vollet, L. R. Pickering, H. L. Dupont, D. L. Walters, and S. Kohl.** 1978. Rotavirus in travelers' diarrhea: study of an adult student population in Mexico. J. Infect. Dis. **137**:324–327.
2. **Flewett, T. H., and G. N. Woode.** 1978. The rotaviruses. Arch. Virol. **57**:1–23.
3. **Goyal, S. M., C. P. Gerba, and J. L. Melnick.** 1978. Prevalence of human enteric viruses in coastal canal communities. J. Water Pollut. Control Fed. **50**:2247–2256.
4. **Hejkal, T. W., C. P. Gerba, and V. C. Rao.** 1982. Reduction of cytotoxicity in virus concentrates from environmental samples. Appl. Environ. Microbiol. **43**:731–733.
5. **Hejkal, T. W., F. M. Wellings, A. L. Lewis, and P. A. LaRock.** 1981. Distribution of viruses associated with particles in wastewater. Appl. Environ. Microbiol. **41**:628–634.
6. **Irving, L. G., and F. A. Smith.** 1981. One-year survey of enteroviruses, adenoviruses, and reoviruses isolated from effluent at an activated-sludge purification plant. Appl. Environ. Microbiol. **41**:51–59.
7. **Kapikian, A. Z., H. W. Kim, R. G. Wyatt, W. L. Cline, J. L.**

Arrobio, G. D. Brandt, W. J. Rodriguez, D. A. Sack, R. M. Chanock, and R. H. Parrott. 1976. Human reovirus-like agent associated with "winter" gastroenteritis. N. Engl. J. Med. **294**:965–972.

8. **Kim, H. W., C. D. Brandt, A. Z. Kapikian, R. G. Wyatt, J. O. Arrobio, W. J. Rodriguez, R. M. Chanock, and R. H. Parrott.** 1977. Human reovirus-like agent infection occurrence in adult contact of pediatric patients with gastroenteritis. J. Am. Med. Assoc. **238**:404–407.
9. **Lund, E., C. E. Hedstrom, and O. Strannegard.** 1966. A comparison between virus isolation from sewage and from fecal specimens from patients. Am. J. Epidemiol. **84**:282–286.
10. **McNulty, M. S.** 1978. Rotaviruses. J. Gen. Virol. **40**:1–18.
11. **Melnick, J. L.** 1947. Poliomyelitis virus in urban sewage in epidemic and in non-epidemic times. Am. J. Hyg. **45**:240–253.
12. **Melnick, J. L., J. Emmons, J. H. Coffey, and H. Schoof.** 1954. Seasonal distribution of coxsackie viruses in urban sewage and flies. Am. J. Hyg. **59**:164–184.
13. **Melnick, J. L., H. A. Wenner, and C. A. Phillips.** 1980. Enteroviruses, p. 529–602. *In* E. H. Lennette and J. J. Schmidt (ed.), Diagnostic procedures for viral, rickettsial, and chlamydial infections, 5th ed. American Public Health Association, New York.
14. **Pang, Q. F., F. X. Qiu, F. R. Yu, S. Z. Chen, Y. Q. Suo, G. Y. Li, Z. M. He, and B. Y. Zhang.** 1980. Studies on the etiology of autumnal infantile acute gastroenteritis-rotavirus. Chin. Med. J. **93**:36–40.
15. **Rubenstein, A. S., and M. F. Miller.** 1982. Comparison of an enzyme immunoassay with electron microscopic procedures for detecting rotavirus. J. Clin. Microbiol. **15**:938–944.
16. **Sack, R. B., H. L. Dupont, B. Rowe, S. L. Gorbach, N. R. Blacklow, S. B. Formal, R. L. Guerrant, A. Z. Kapikian, D. A. Sack, and L. R. Trabulsi.** 1978. International conference on the diarrhea of travelers—new directions in research: a summary. Etiology—summary of session. J. Infect. Dis. **137**:358–360.
17. **Sharp, D. G., R. Floyd, and J. D. Johnson.** 1975. Nature of the surviving plaque-forming unit of reovirus in water containing bromine. Appl. Microbiol. **29**:94–101.
18. **Smith, E. M., and C. P. Gerba.** 1982. Development of a method for detection of human rotavirus in water and sewage. Appl. Environ. Microbiol. **43**:1440–1450.
19. **Steinhoff, M. C.** 1980. Rotavirus: the first five years. J. Pediatr. **96**:611–622.
20. **Steinmann, J.** 1981. Detection of rotavirus in sewage. Appl. Environ. Microbiol. **41**:1043–1045.
21. **Sutmoller, F., R. S. Azeredo, M. D. Lacerda, O. M. Barth, H. G. Pereira, E. Hoffer, and H. G. Schatzmayer.** 1982. An outbreak of gastroenteritis caused by both rotavirus and *Shigella sonnei* in a private school in Rio de Janeiro. J. Hyg. **88**:285–293.
22. **Von Bonsdorff, C. H., T. Hovi, P. Mäkelä, and A. Mörttinen.** 1978. Rotavirus infections in adults in association with acute gastroenteritis. J. Med. Virol. **2**:21–28.
23. **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.
24. **Wellings, F. M., A. L. Lewis, C. W. Mountain, and L. Stark.** 1975. Virus consideration in land disposal of sewage effluents and sludge. Fla. Sci. **38**:202–207.