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Detection of Rotavirus in Sewage

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For detection of rotavirus, domestic sewage was concentrated by two different methods: (i) adsorption to and elution from positively charged Seitz filters, followed by ultracentrifugation, and (ii) chemical precipitation. The concentrated fluids were tested by an enzyme-linked immunosorbent assay and electron microscopy. In 6 of 24 (25%) samples, rotavirus was detectable after the combined filtration and ultracentrifugation technique with both an enzyme-linked immunosorbent assay and electron microscopy. No positive results were obtained after chemical precipitation.

Viral gastroenteritis is a common disease of short duration characterized by diarrhea and vomiting. Rotavirus has been shown to be an important cause of this disease in infants and children in many parts of the world, especially in winter (6). In adults, rotavirus infections associated with acute gastroenteritis have also been reported (13). In developed areas it is a common cause of hospitalization and can be fatal (1). In underdeveloped countries with poor sanitation and nutrition, rotavirus infections are a major cause of infant morbidity and mortality.

Since a large number of rotaviruses are excreted with feces, they may be detected in sewage and sewage-contaminated waters. Outbreaks of waterborne diseases with suspected rotavirus etiology have recently been reported (8, 10), giving increasing evidence that these viruses can be transmitted through the consumption of sewage-polluted waters. Using simian rotavirus SA11 as a model for human rotavirus, studies on the stability of rotavirus have shown that the infectivity is stable and that these viruses can survive in natural waters for a long time (2, 4).

Since human rotavirus does not grow efficiently in cell culture systems (14), no reports on the concentration and recovery from sewage and contaminated waters have been made.

An investigation was started to determine the prevalence of rotavirus in domestic sewage with methods independent of the cultivation of these viruses and based on direct evidence with an enzyme-linked immunosorbent assay (ELISA) and electron microscopy (EM). In this report the successful detection of rotavirus in sewage is described.

Domestic sewage from the city of Kiel was collected at different times from 13 June 1980 to 24 July 1980 and concentrated by two different methods. With the filter technique, 2 liters of sewage were filtered through positively charged asbestos-cellulose Seitz filters (Seitz-Filter EKS, Ø 14 cm, Bad Kreuznach, Germany), followed by an elution with 50 ml of 3.0% beef extract, pH 9.0. The eluate was centrifuged in an L8-70 ultracentrifuge (Beckman Instruments, Inc., Fullerton, Calif.) with a 70.1 Ti rotor for 4 h at 40,000 rpm. The supernatant was discharged, and the sediment in one-half of the tubes was resuspended in 0.5 ml of ELISA diluent, containing 0.15 M phosphate-buffered saline (pH 7.2), 4% Tween 20, and 2.5% bovine serum albumin. The other half was resuspended in 0.5 ml of 3% beef extract (pH 9.0) effecting a 2,000-fold concentration. Using chemical precipitation, 200 mg of $Al_2(SO_4)_3 \cdot 18H_2O$ was added to 1 liter of sewage, and the pH was reduced by 0.1 N HCl to 5.4 to 5.6. On the next day the supernatant was discharged, and one-half of the sediment was resuspended in 5.0 ml of ELISA diluent. The other half was suspended in 5.0 ml of 3% beef extract, pH 9.0, effecting a 100-fold concentration.

Two different methods were used for the detection of rotavirus in the concentrated samples. First, a commercially available ELISA (Behringwerke, Marburg, Germany) was chosen for the sample in the ELISA diluent. Results of this assay were read at 405 nm with a colorimeter (Titertek Multiskan). Samples were considered positive if the extinction was two times greater than the extinction of the negative control samples.

The beef extract solution was further concentrated for electron-microscopic studies by the agar-diffusion-filtration method as described by Kelen et al. (7). Briefly, a microdrop of about 0.025 ml was deposited on the surface of an agar-coated slide (Oxoid Ionagar no. 2). A Formvar-coated grid was placed immediately upside down

and left floating on top of the drop. The fluid phase of the drop diffused into the agar layer, and grids settled on the surface of the agar were negatively stained with 2% potassium phosphatungstate solution (pH 6.5) and examined at a magnification of 40,000 for the presence of rotavirus particles.

The study shows that in 6 of 24 (25%) samples from domestic sewage, rotavirus could be detected by both ELISA and EM after the filter technique and ultracentrifugation of the eluate (Table 1). There were no cases in which a sample was positive for rotavirus in only one of the two test systems. After flocculation, no rotavirus could be detected in the 24 samples with either method.

Since human rotavirus cannot be readily propagated in cell culture systems, suitable concentration methods are not, in general, available. In the future, simian rotavirus SA11 may be a useful model for developing techniques to detect rotavirus particles in a low concentration in sewage and sewage-contaminated waters. In our study, techniques were used that have been successfully applied for the concentration of enterovirus, such as filtration through positively charged filters (12) and chemical precipitation by inorganic salts. Using these procedures, 2,000- and 100-fold concentrations were achieved for the filter technique and chemical precipitation, respectively. This 20-fold concentration difference may account for the failure of the chemical precipitation technique to detect rotavirus. However, the possibility that this failure may result from the different physicochemical behavior of these viruses in the concentration procedure cannot be excluded. Experiments with another method, the talc-Celite process, had shown that both poliovirus and simian rotavirus could be concentrated with equal efficiency (11). However, when the adsorption of enterovirus and rotavirus to aluminium hydroxide and activated sludge flocs were compared, it was discovered that relatively small amounts of rotavirus were absorbed in relation to the amounts of poliovirus. Different techniques have been developed that can detect rotavirus antigens directly in clinical specimens, including radioimmunoassay (5), immunofluorescence (9), ELISA (15), complement fixation (16), and electron microscopy. For the determination of rotavirus in sewage, two methods were chosen which are utilized in this laboratory for clinical specimens. ELISA is as sensitive as EM for the detection of rotavirus from samples of concentrated sewage. This is in agreement with the determination of rotavirus in human feces (15). With EM, rotavirus particles can be identified easily on the basis of their size and morphology. ELISA is simple to perform and does not require sophisticated technical equipment, making it suitable for large-scale examinations.

TABLE 1. *Detection of rotavirus in sewage, using two different methods of sample concentration and virus determination*

Date of sample (1980)	Seitz-filter method		Flocculation method	
	ELISA	EM	ELISA	EM
13 June	−	−	−	−
16 June	−	−	−	−
17 June	+	+	−	−
18 June	−	−	−	−
20 June	−	−	−	−
23 June	+	+	−	−
24 June	+	+	−	−
25 June	−	−	−	−
26 June	−	−	−	−
27 June	−	−	−	−
30 June	+	+	−	−
1 July	+	+	−	−
2 July	−	−	−	−
3 July	−	−	−	−
7 July	−	−	−	−
9 July	−	−	−	−
10 July	−	−	−	−
11 July	−	−	−	−
15 July	−	−	−	−
17 July	−	−	−	−
18 July	−	−	−	−
21 July	−	−	−	−
23 July	+	+	−	−
24 July	−	−	−	−

The main limitation of both methods relates to the high concentration of virus particles necessary in the samples. Depending on the negative-staining method used, about 10^7 virions per milliliter must be present in the specimen to be detected by EM on the grid. Furthermore, neither method can distinguish between infective and noninfective particles and between rotaviruses from human and animal origins, because all rotaviruses are reactive in ELISA and morphologically indistinguishable by EM. In domestic sewage, rotavirus from animals cannot be expected to reach a concentration of about 10^7 virions per 2 liters of sewage.

In spite of the disadvantages of the methods used for detection of rotavirus, positive findings in 25% of the samples taken during the summer gave preliminary estimates of the occurrence and number of these viruses in sewage, indicating that a transmission through sewage-contaminated waters is possible.

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