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OCCURRENCE OF ROTA- AND ENTEROVIRUSES IN DRINKING AND ENVIRONMENTAL WATER IN A DEVELOPING NATION

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Abstract—Rotaviruses have been implicated as a major cause of childhood and traveler's diarrhea in developing countries. Since water is known to be a vehicle of transmission of other enteric viruses, we sought to determine if water could play a role in the transmission of rotavirus infections in a developing nation by applying recently developed techniques for the concentration of viruses from tapwater and environmental (lake, river, ocean and aqueduct) water in Mexico. In an initial survey during the rainy season in August 1978, rotavirus was detected in 10 of 10 drinking water samples and coxsackie B4 or B6 virus in 5 of 10. In a larger survey during the dry season in December 1979, rotavirus was recovered from 3 and enteric viruses from 8 of 21 drinking water samples. Water quality data, available for the 1979 survey, indicated that while many tapwater samples did not meet U.S. coliform standards, some samples containing infectious virus did. Our data suggest that current bacteriological water quality standards for potable water do not reflect viral contamination.

Key words—rotavirus, drinking water, water treatment, enteroviruses, coliforms, standards

INTRODUCTION

Evidence of rotavirus infection is frequently found among travelers to developing countries (Bolivar *et al.*, 1978; Keswick *et al.*, 1982). the epidemiology of human rotavirus infections is incompletely understood, but person-to-person transmission has been implicated by studies showing intrafamily spread (Kim *et al.*, 1977; Wenman *et al.*, 1979). Among travelers, spread from infants, with whom there is generally little contact, seems unlikely. Transmission via food, which has been implicated in enterotoxigenic *Escherichia coli* infections of travelers (Foster *et al.*, 1980; Tjoa *et al.*, 1977) has not been examined or not found to be epidemiologically linked. Excretion of large amounts of rotavirus in the feces of infected individuals and its consequent presence in sewage indicate that this virus may also be waterborne (Smith and Gerba, 1982). The present study was undertaken to determine if water may serve as a transmission vehicle for rotavirus. Because disease expression in adults is frequently mild or asymptomatic and the concentration of viruses in potable water is expected to be low, epidemiologic studies would have required too many subjects to be practical for a preliminary study (Melnick *et al.* 1978). Instead we applied recently developed techniques for the concentration and detection of viruses from large volumes of water to the investigation of the presence of rotavirus in drinking and environmental water (Smith and Gerba, 1982; Gerba *et al.*, 1978).

Several investigators have reported the isolation of enteroviruses in drinking water, and coxsackie virus B has been recovered from environmental water at the time of an outbreak (World Health Organization, 1979; Hejkal *et al.*, 1982). Waterborne outbreaks of gastroenteritis implicating rotavirus or Norwalk-like agents have been described (Kaplan *et al.*, 1982), but to our knowledge the recovery of rotavirus from drinking water has not been previously reported. This report describes the detection of rotavirus and enteroviruses in drinking and environmental water in Mexico along with data on water quality.

MATERIALS AND METHODS

Water samples

Ten drinking water samples from sites throughout the city of Guadalajara and one sample from the canal supplying the city water treatment plant were collected in August, 1978. The volume of all samples was 20 l. In December 1979, 21 drinking water samples were collected from sites throughout the cities of Guadalajara, Chapala, surrounding towns, and Puerto Vallarta along with six environmental samples including one city's water supply source. Seven samples were also collected after each treatment stage at the water treatment plant in Guadalajara. Again, the volumes of all samples except two were 20 l. Concentrates were frozen, transported on dry ice and stored at −70 C until assayed.

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At the time samples were collected in 1979, portions were assayed for free and total chlorine using a Hach Chlorine Test Kit (Ames, IA). Portions were assayed for total and fecal coliforms according to standard methods using the membrane filter technique (APHA, 1980). Turbidity was determined on samples transported on ice back to the United States using a turbidimeter (Model 2100A, Hach Chemical Co., Ames, IA).

Concentration methods

Residual free chlorine in the 20-l. drinking water samples was neutralized at the time of collection by the addition of 10 ml of a 1% solution of sodium thiosulfate. The pH was adjusted to 3.5 with 1 N HCl, and aluminum chloride ($AlCl_3$) was added to a final concentration of 0.0005 M (Gerba *et al.*, 1978). The sample was then passed through a 3 and 0.45 μm nominal pore size fiberglass-epoxy and melamine impregnated 142 nm diameter disc filter (Duo-Fine Series, Filterite, Timonium, MD) in series. The adsorbed virus was eluted for 2 min with 50 ml of 0.05 M glycine–NaOH at pH 10.0 containing 10% tryptose phosphate broth (Difco, Detroit, MI). Samples were then neutralized by the addition of 1 N HCl and frozen. Prior to assay eluents were further concentrated either by hydro-extraction using PEG (polyethylene glycol) or by ultracentrifugation to a final volume of 1.5 ml each (Gerba *et al.*, 1978).

Virus detection

Samples (0.25–0.4 ml) for rotavirus detection were plated on a continuous line of fetal rhesus monkey kidney cells (MA-104) grown to confluence on 15 mm circular coverslips in 16 mm tissue culture wells, 24 wells/plate. Pancreatin (0.03 ml) of a 1:60 dilution from stock, (Gibco, Grand Island NY) was added and the tissue culture plates were centrifuged at 200 *g* for 1 h at 23°C (IEC UV). The samples were removed, the monolayers washed with saline, and incubated for 48 h at 37°C. At the end of 48 h, the coverslips were removed, fixed for 10 min in 20°C ethanol and stored for assay by indirect immunofluorescence. Uninfected control coverslips were interspersed in wells between the samples, and no positive controls (SA-11 or human rotavirus) were assayed on plates containing environmental samples in order to avoid the possibility of cross contamination. Antiserum raised in guinea pigs against SA-11 was shown to be specific by neutralization and indirect immunofluorescence techniques (Estes *et al.*, 1979). Reactivity was checked with reovirus types 1–3, poliovirus type 1, echovirus type 1 or coxsackievirus B4 and B6. All reactivity for the commercially obtained FITC-conjugated rabbit anti-guinea pig IgG (Microbiological Associates, Bethesda, MD) to SA-11 infected MA-104 cells was completely removed by absorption with mouse liver powder and SA-11 infected cells. The entire coverslip from samples and controls was scanned using a Zeiss Universal fluorescent microscope and the number of brightly staining cells were counted. No fluorescent foci were found on uninfected control coverslips.

Samples (0.5 ml) for enterovirus detection were assayed on BGM (Buffalo Green Monkey) cells and examined daily for 10 days for plaques or cytopathic (CPE) effect. All CPE positive cultures and picked plaques were passed once in BGM cells. Isolates were then identified by neutralization with LIM–Benyesh–Melnick pools of cross-reacting antisera (Melnick *et al.*, 1973).

RESULTS

Drinking water isolates August 1978

Table 1 shows the results obtained during the initial sampling during the rainy season. All 10 drinking water samples contained infectious rotavirus as did the water from the canal supplying the treatment plant. The number of foci per 20 l. was calculated from the number of foci in 0.4 ml of the total 1.5 ml volume of the concentrate. A concentration efficiency of approx. 50% for SA-11 is obtained with the methodology we employed but the data are not corrected for this factor (Gerba *et al.*, 1978). Two samples containing rotavirus were subjected to neutralization studies using anti-SA-11 anti-sera and human pre-illness and convalescent anti-rotavirus serum (Smith and Gerba, 1982). Both isolates were neutralized only by the human convalescent sera. Five drinking water samples contained coxsackie viruses B4, B5 or B6 and the canal water contained coxsackie viruses B4 and B6.

Drinking water isolates December 1979

Table 2 shows the results of rotavirus and enterovirus detection in drinking water along with water quality data. Three of 21 drinking water samples contained infectious rotavirus while 8 contained enteroviruses. The frequency of isolation of rotaviruses was higher during the rainy season (100%) than the dry season (14%). Rotavirus was not found in any of 7 environmental samples, 5 of which contained enteroviruses. Two samples were negative for both entero- and rotaviruses. Insufficient quantities of samples containing rotavirus remained to undertake neutralization studies.

In addition to the studies of environmental and drinking waters, virus removal by standard water treatment was also studied (Table 3). A total of 7

Table 1. Virus isolation from drinking water during the rainy season, August 1978

Site	Source	Rotavirus I.F. foci/20 l.	Enterovirus/20 l. Plaques	CPE	Isolate
1	Drinking water, tap	105	0	—	
2	Drinking water, tap	180	0	+	Coxsackie B4
3	Drinking water, tap	68	0	—	
4	Drinking water, tap	26	0	—	
5	Drinking water, tap	112	20	+	Coxsackie B5
6	Drinking water, tap	112	0	—	
7	Drinking water, tap	210	0	+	Coxsackie B6
8	Drinking water, tap	160	0	+	Coxsackie B6
9	Drinking water, tap	37	0	—	
10	Drinking water, final effluent treatment plant	16	27	+	Coxsackie B6
11	Environmental, canal pre-treatment	83	11	+	Coxsackie B4, B6

Table 2. Virus isolation from drinking water during the dry season, December 1979

Source	Rotavirus foci/20 l.	Plaques	Enterovirus/20 l. CPE	Enterovirus/20 l. Identification	Coliforms/100 ml Total	Coliforms/100 ml Fecal	Chlorine ($mg\,l^{-1}$) Free	Chlorine ($mg\,l^{-1}$) Total	Turbidity NTU	pH
Tap, city	0	0	—	—	0	0	0	0	0.17	7.7
Bottled water	0	0	—	—	0	0	0	0.06	0.14	7.5
Tap, city	0	0	—	—	10	0	0.06	0.05	0.24	7.4
Tap, village	0	0	—	—	0	0	0.04	0.06	0.16	7.1
Artesian well	112	0	—	—	0	0	0	0	0.16	7.2
Tap, private home	0	0	—	—	0	0	0.09	0.18	0.55	7.6
Deep well	0	0	—	—	2	0	0.08	0.12	4.0	7.7
Bottled water, commercial	0	0	+	Coxsackie B3	1	0	0	0.04	0.25	7.7
Ice, hotel ice maker	0	0	+	Coxsackie B3	0	0	0.02	0.07	0.61	6.7
Tap, market	37	0	—	—	5	0	0.06	0.06	0.24	6.9
Tap, gas station no. 1	0	0	—	—	0	0	0.06	0.18	0.84	7.6
Tap, hotel*	2500	0	—	—	1	0	0.26	0.50	0.47	7.0
Tap, gas station no. 2	0	0	—	—	3	0	0	0	0.15	6.8
Tap, town no. 1 on lake	0	2	—	Not typed	ND	3.3×10^2	0	0	1.1	7.2
Tap, town no. 2 on lake	0	0	—	—	0	0	0.1	0.3	1.29	7.6
Tap, city	0	0	—	—	0	0	0	0	0.62	7.5
Tap, gas station, lake	0	0	+	Coxsackie B3	14	0	0	0	0.13	7.8
City tap, market	0	—	+	Not typed	3.0×10^3	2.0×10^3	0.03	0.08	0.30	6.9
Gas station	0	2	—	Coxsackie B3	TNTC	1.0×10^3	0	0.04	0.31	7.5
Tap, hotel	0	—	+	Not typed	0	0	0.02	0.06	0.20	7.0
Bottled water	0	—	+	Coxsackie B3	20	0	0.02	0.04	0.14	6.8

ND = no data; TNTC = too numerous to count; *40 l. sample.

Table 3. Virus isolation after major water treatment process

Source	Rotavirus foci/20 l.	Plaques	Enterovirus/20 l. CPE	Enterovirus/20 l. Identification	Coliforms/100 ml Total	Coliforms/100 ml Fecal	Chlorine ($mg\,l^{-1}$) Free	Chlorine ($mg\,l^{-1}$) Total	Turbidity NTU	pH
Canal, plant intake	0	0	—	—	1.7×10^6	ND	0	0	10.0	8.0
Canal, plant intake*	0	—	+	Coxsackie B3	ND	ND	ND	ND	13.0	ND
Post-clarification (Superfloc, $Al_2(SO_4)_3$)	0	0	—	—	0	0	1	1.1	1.4	7.7
Pre-filtration	0	0	—	—	0	0	0.16	1.0	0.97	8.5
Post-filtration	0	2	+	Coxsackie B3	0	0	0.6	1.7	1.0	7.6
Finished water	0	0	—	—	0	0	2.4	2.5	1.0	7.6
Finished water	0	0	+	Cocsackie B3	0	0	0.24	0.40	1.4	ND

ND = no data; *16-l. sample.

samples were collected after each major treatment stage (clarification, filtration and chlorination) at a $9 m^3 s^{-1}$ (205 mgd) water treatment plant. No samples were positive for rotavirus, however, coxsackie virus B3 was detected in the raw water, in one sample after sand filtration and in one chlorinated finished water sample.

Of the dry season water samples containing viruses, only 2 met currently recommended chlorine levels (*Federal Register*, 1975) (residual chlorine $0.2 mg l^{-1}$) (Table 2). One of these samples contained 1 coliform per 100 ml. A total of 11 of 21 drinking water samples exceeded coliform standards, 8 contained viruses. Significantly, 3 samples in which no coliform bacteria were detected were found to be positive for virus. Only the two finished water samples at the water treatment plant (Table 3) and one other (Table 2) met both coliform standards and chlorine recommendations and one of these samples was found to be positive for virus.

Rotaviruses were found in one sample (Table 2) meeting bacteriological standards, however, this sample was from an unchlorinated well. High numbers of rotavirus were detected in one sample (Table 2) which contained free and total residual chlorine and only 1 total coliform per 100 ml. The enterovirus virus positive finished water sample (Table 3) which met both bacteriological and chlorine standards, but not turbidity standards, was negative for rotavirus. Over all, enteric viruses were detected in 3 samples meeting coliform standards, one meeting the chlorine recommendations and in the one sample meeting both.

All but two drinking water samples met U.S. turbidity standards of one or less turbidity units (*Federal Register*, 1975).

DISCUSSION

Recently, methods to detect rotavirus and enterovisuses in potable and environmental water have been developed. Using these methods, enteric viruses have been isolated from seawater, groundwater, well water, treated sewage and tapwater, while rotavirus has been recovered from sewage (Gerba *et al.*, 1978). Such studies have occasionally shown that current bacteriological standards of water quality do not indicate the presence of pathogenic viruses. This study represents the first report of the recovery of rotavirus from drinking water. Neutralization studies on two isolates strongly suggest that they are, or are very closely related to, human rotavirus. The presence of enteroviruses in drinking water has been reported from several countries and our findings extend those reports (World Health Organization, 1979), the results also suggest that a possible source of travelers' diarrhea in developing nations is drinking water. The mean frequency of virus isolation from drinking water was lower in the second survey during the dry season. Several factors may account for this variation. For example, water quality is known to influence the presence of viruses in water (World Health Organization, 1979). Unfortunately, water quality data was not obtained on the initial samples, so direct comparison of the two studies by these criteria is not possible, however, follow up studies have indicated that the rainy season is associated with a dramatic decrease of raw water quality due to the large amounts of sediment produced by storm runoff. The rainy season, which in some areas correlates with an increased incidence of gastroenteritis, extends into August in the study area while December is a "dry" month. However, seasonal variation in rotavirus infection is much less pronounced in temperate areas than in the northern latitudes where is is predominantly a winter viral infection. In contrast, increased numbers of rotavirus infections in other areas have been associated with dry season periods of low relative humidity (Paul and Erinle, 1982). Further research into the epidemiology of rotavirus infection is needed to clarify the relationship of water, climate and population factors such as crowding to transmission. In addition to water quality and seasonal factors, variations in techniques between studies also may have resulted in lower recovery rates. The samples in the first study were finally concentrated by hydroextraction while those in the second study were finally concentrated by ultracentrifugation. The relative efficiencies of these two methods for human rotavirus is under study.

The source of rotaviruses and enteroviruses in environmental and tap water in the areas we studied is not precisely known, however opportunity exists for human sewage contamination of the environmental water sources in one of the areas studied from a large unsewered population upstream of the water treatment plant. We found enteroviruses in the lake supplying the city water, as well as in the canal carrying water to the treatment plant (Tables 1, 2 and 3). Our preliminary data suggest that enteroviruses can penetrate and survive through a modern, well functioning water treatment plant (flocculation, sand filtration and chlorination) and that viruses can be recovered from water that has acceptable bacteriological and chlorine levels (U.S. EPA, 1975) coliforms 0/100 ml and free chlorine $0.2 mg l^{-1}$. The high turbidity of the water supply indicates significant amounts of suspended solids which may render routine purification processes ineffective as viruses may be stabilized by adsorbing to suspended matter (World Health Organization, 1979). In the tapwater samples obtained at some distance along the water distribution system the potential for post treatment contamination exists, especially where rooftop cisterns are used to supplement supply during the dry season.

Post-treatment contamination is compounded where levels of residual chlorine are insufficient to inactivate bacteria and viruses. Rotaviruses and enteroviruses are resistant to chlorine and may persist

for extended periods under a variety of environmental conditions (Melnick *et al.*, 1978).

Our study was not designed to determine the role of waterborne human viruses in causing disease in humans. Insufficient data exist with regard to minimum infective dose, route of infection (respiratory vs gastrointestinal), and other determinants of infection to draw conclusions regarding the public health hazard of these viruses to be drawn. Nevertheless, water has been implicated epidemiologically in outbreaks of gastroenteritis of suspected rotavirus viral etiology (Lycke *et al.*, 1978; Morens *et al.*, 1979; Zamotin *et al.*, 1981; Sutmoller *et al.*, 1982). Furthermore, it is fairly well established that other enteric viruses can be transmitted by water and will cause human disease, ranging from mild eye and respiratory infections to diarrhea to severe hepatitis (World Health Organization, 1979; Sanchez *et al.*, 1982; Hejkal *et al.*, 1982). Because rotavirus can also cause life-threatening disease (in infants), it seems reasonable to consider waterborne rotavirus a public health hazard. The data in this report showing infectious rotavirus in potable and environmental waters confirms the potential of the virus for waterborne transmission. Furthermore, the finding of infectious virus in water samples meeting U.S. quality standards suggests that current water quality standards and treatment processes need to be re-evaluated.

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