Epidemiology

DIARRHEA DUE TO ROTAVIRUS AND PROBABILITY OF SEWAGE CONTAMINATION

S. A. KHAN*
AQEEL AHMED*
SYED M. KHALID*

SUMMARY : Rotavirus, the second major cause of gastroenteritis in infants and children has been investigated in 166 diarrheal disease patients and 10 sewage samples obtained from different localities of Karachi. It was observed that highest incidence (40%) of rotavirus occur in infants up to one year and the incidence decrease with age. In general, the incidence of rotaviral infection is significantly high among infants and children up to 5 years of age as compared with 16.6% for adults. Evidence has been presented about the presence of rotavirus in 60% sewage samples analyzed.

Key Words: Rotavirus, diarrhea.

INTRODUCTION

Acute viral gastroenteritis is a very common illness (1), that occurs in both endemic and epidemic forms (2). It affects all age groups world-wide and is also responsible for some of the commonly caused travelers diarrhea. The clinical manifestations of this illness is variable, but in general it is self limiting, has an explosive onset and is manifested by varying combinations of diarrhea, nausea, vomiting, low grade fever, abdominal cramps, headache, anorexia, myalgia and malaise. It is not only responsible for a great deal of misery and time loss from school and work, but can be severe, indeed fatal, in infants and elderly and debilitated patients due to the associated malabsorption, may trigger or enhance the morbidity associated with malnutrition or marginally nourished population.

Up till now, only two viruses, Rotavirus and Norwalk virus, are recognized as medically important etiological agents of human gastroenteritis. Among the two, only rotavirus has been successfully propagated in cell cultures. Many aspects of its antigenic nature (3), basic virology, epidemiology and pathogenesis have been elucidated. The incidence of rotaviral diarrhea in this part of the world is unknown and therefore warrants urgent attention.

In this paper, we studied the rate of incidence of rotaviral diarrhea among a series of patients. We also tried to establish the possible source of infection in Karachi City.

* From Department of Microbiology, University of Karachi, Karachi-75270, Pakistan.

MATERIALS AND METHODS

Collection of stool sample

Stool samples were collected from patients with diarrhea, belonging to different localities in Karachi, in sterile disposable plastic tubes. The specimen were frozen at -20°C, until sufficient number of samples were received and analyzed for the presence of rotavirus antigen by the Elisa technique.

Separation and concentration of rotavirus from sewage sample

Raw sewage samples were collected in clean and sterile bottles. The samples were placed in dialysis tubings and were covered all over with polyethylene glycol 4000 (PEG-4000) and left over night in a refrigerator for hydro-extraction. The concentrated sewage sample was collected by washing the dialysis tubing with 15 ml of phosphate buffered saline (pH 7.4).

The viruses were eluted from the organic matter by adjusting the pH to 10 using 1 N NaOH. The sample was then centrifuged at 1000 g for 15 minutes to remove suspended material. The pH of the supernatant was then lowered to 7 with 1 N HCl and analyzed for the presence of rotavirus antigen by the Elisa technique.

Detection of rotavirus antigen by ELISA

The presence of rotavirus antigen was detected in human fecal samples and in influents (Raw sewage) using ROTAZYME®II Diagnostic kit, (Abbott laboratories, North Chicago, USA).

RESULTS AND DISCUSSION

Presence of rotaviruses were detected in 51 out of 166 fecal samples collected from diarrheal patients residing in different localities in Karachi (Table 1). Majority of the cases (30 out of 75) were positive among the infants in the age group of up to 1 year. Followed by 16 positive cases out of 51 cases were among the children in the age group between 1-5 years. Four out of 35 cases were positive in persons of 6-15 years age group. Only 1 case out of 6 had shown the presence of rotaviral diarrheal in persons above 15 years of age.

Rotaviruses are one of the major causes of gastroenteritis in infants and in young children in many parts of the world (4). Adults are less likely to become infected with rotavirus than children (5-8) and infected adults are also more frequently asymptomatic than children (9).

Most of the positive cases were from the persons living in the remote areas, where increased family size and over crowding is a common factor. These are reportedly significant risk factors for the mode of transmission of rotavirus due to environmental contamination, contact with the hands of infected persons (11).

The role of water in the transmission of viruses is well established. However, the epidemiological evidence of water-borne transmission of human viruses is limited to only few including rotavirus (12-15).

Epidemiological data available for water-borne outbreaks due to enteroviruses and other enteric viruses may fail to reflect a complete picture of the role of water in the transmission of viral diseases for several reasons. Many of these viruses cause unapparent infection particularly in children. In such cases long incubation periods contribute to difficulties in recognition and investigation of outbreaks. Furthermore, epidemiological techniques are not sufficiently sensitive to detect low-level transmission of virus diseases through water (16-19).

The quality of water consumed in different parts of the world varies considerably. Conventionally treated and disinfected water is supplied in major cities of the western world. Untreated water from canals, streams and rivers is often a source of drinking water in small towns and villages in developing countries and in Pakistan as well. The virological quality of these waters is unknown. Thus, the data concerning the presence of viruses in drinking water are available only for conventionally purified and chlorinated water (20, 21).

Explosive outbreaks of viral gastroenteritis resulting from sewage contamination of water supplies have been well documented (22). Present work has been designed to examine whether or not rotaviruses were present in the influents, to evaluate virological quality of water as delivered to the consumers in different localities of Karachi. The results show the presence of rotavirus in 60% influent samples (Table 2). It was also found that some of the tap-water samples collected, had shown the presence of fecal coliforms (results not shown), suggesting that the water source was also polluted due either to the intermittent water supply or to a leakage in the distribution pipeline and in the nearby sewage lines. Because of these factors, drinking water must be regarded as having a very significant potential as a vehicle for the transmission of rotaviruses.

Table 1: Detection by Elisa of rotaviruses from the fecal samples.

Age group (in years)	No. of samples	No. of rotavirus-positive specimens (%)
0-1	75	30 (40)
1-5	51	16 (31.37)
5-15	35	4 (11.43)
Above 15	06	1 (16.6)
Total	166	51 (30.72)

Table 2: Detection by Elisa of rotaviruses antigen in Karachi sewage.

Sample	Rotavirus
K_1	positive
K_2	negative
K_3	negative
K_4	positive
K_5	positive
K_6	positive
K_7	negative
K_8	positive
K_9	negative
K_{10}	positive

Furthermore, although the coliform tests were generally seemed to be reliable for the virological safety of treated drinking water supplies but has been questioned because some viruses are more resistant than *Escherichia coli* to a variety of adverse environmental conditions and water treatment processes including chlorination (20). Viruses were detected in drinking-water supplies which met conventional coliform specification tests (20, 23-28). In addition, a proportion of water samples taken during some water-borne viral

outbreaks yielded negative results for coliform tests which indicates that under certain circumstances coliforms may fail to identify a viral hazard (13, 26). Therefore, the most reasonable approach for controlling the transmission of viruses through drinking-water is to recommend consistently meeting the treatment criteria by including additional parameters.

REFERENCES

1. Blacklow NR, Dolin R, Fedson DS, DuPont H, Northrop RS, Hornick RB, Chanock RM : Acute infectious nonbacterial gastroenteritis: etiology and pathogenesis. Ann Intern Med, 76:993-1008, 1972.

2. Balcklow NR, Cukor G : Viral gastroenteritis. N Engl J Med, 304:397-406, 1981.

3. Bridger JC, Clarke IN, McCrae MA : Characterization of an antigenically distinct porcine rotavirus. Infect Immun, 35:1058-1062, 1982.

4. Kapikian AZ, Greenberg HB, Wyatt RG, Kalica AR, Kim HW, Brandt CD, Rodriguez WJ, Parrot RH, Chanock RM : Viral gastroenteritis, pp 283-326. In AS Evans (ed), Viral infections of humans, 2nd ed. Plenum Publishing Corp, New York, 1982.

5. Bryden AS, Davies HA, Hadley RE, Flewett TH, Morris CA, Oliver P : Rotavirus enteritis in the west midlands during 1974. Lancet, 2:241-243, 1975.

6. Kapikian AZ, Kim HW, Wyatt RG, Cline WL, Arrobio JO, Brandt CD, Rodriguez WJ, Sack SA, Chanock RM, Parrott RH : Human rotavirus-like agent as the major pathogen associated with winter gastroenteritis in hospitalized infants and young children. N Engl J Med, 294:965-972, 1976.

7. Bishop RF, Cameron DJ, Veenstra AA, Barnes GL : Diarrhoea and rotavirus infection associated with differing regimens for post natal care of newborn babies. J Clin Microbiol, 9:525-529, 1979.

8. Chrystie IL, Totterdell BM, Banatvala JE : Asymptomatic endemic rotavirus infections in the newborn. Lancet, 1:1176-1178, 1978.

9. Wenman WM, Hinde D, Feltham S, Gurwith M : Rotavirus infection in adults: results of a prospective family study. N Engl J Med, 301:303- 306,1979.

10. Gurwith M, Wenman W, Gurwith D, Branton J, Feltham S, Greenberg H : Diarrhea among infants and young children in Canada: a longitudinal study in three northern communities. J Infect Dis, 147:685-692, 1983.

11. Samadi AR, Huq MH, Ahmad QS : Detection of rotavirus in hand washings of attendants of children with diarrhea. Brit Med J, 286:188,1983.

12. Craun GF : Outbreaks of water-borne disease in the United States: 1975-1976. J Am Wat Wks Ass, 71:422-428, 1981.

13. Morse LJ, Bryan JA, Hurley JP, Murphy JF, O'Brien TF, Wacker WEC : The Holy Cross college football team hepatitis outbreak. J Am Med Ass, 219:706-708, 1972.

14. Appleten H : Outbreaks of viral gastroenteritis associated with the consumption of shellfish. In viruses and wastewater treatment. Ed by M Goddard, M Bulter. Pergamon Press, pp 287-289, Oxford, 1981.

15. Hejkal TW, Keswick B, LaBelle RL, Gerba CP, Sanchez Y, Dreesman G, Hafkin B, Melnick JL : Viruses in community water supply associated with an outbreak of gastroenteritis and infectious hepatitis. J Am Wat Wks Ass, 74:318-321, 1982.

16. Grabow WOK : Review paper. The virology of waste-water treatment. Water Res, 2:675-701, 1968.

17. Melnick JL, Gerba CP, Wallis C : Viruses in water. Bull Wld Hlth Org, 56:499-508, 1978.

18. Mosley JW : Transmission of viral diseases by drinking water. In transmission of viruses by the water route. Ed by G Berg, pp 5-23. Interscience, New York, 1967.

19. WHO Scientific Group : Human viruses in water, waste-water and soil, Technical Report Series 639, p 50, World Health Organization, Geneva, 1979.

20. Melnick JL, Gerba CP : Viruses in surface and drinking waters. Env Intl, T:3-7,1982.

21. Payment P, Trudol M, Plante R : Elimination of viruses and indicator bacteria at each step of treatment during preparation of drinking water at seven water treatment plants. Appl Env Microbiol, 49:1418-1428, 1985.

22. Craun GF, McCabe LJ : Review of the causes of waterborne disease outbreaks. J Am Wat Wks Ass, 65:74-84, 1973.

23. Berg G, Metcalf TG : Indicators of viruses in waters. In Indicators of viruses in water and food. Ann Arbor Science, pp 267-296, Ann Arbor, 1978.

24. Committee Report : Viruses in drinking water. J Am Wat Wks Ass, 71:441- 444, 1979.

25. Committee Report : Water-borne disease in the United States and Canada. J Am Wat Wks Ass, 73:528-529, 1981.

26. Craun GF : Impact of Coliform standard on the transmission of disease. In Evaluation of the Microbiology Standards for Drinking water, Ed by CW Hendricks, pp 21-35. US Environmental Protection Agency, Washington, DC, 1978.

27. Haley CE, Gunn RA, Hughes JM, Lippy EC, Craun GF : Outbreaks of water-borne disease in the United States: 1978. J Infect Dis, 141:794-797, 1980.

Correspondence:
Shakeel Ahmed KHAN
Department of Microbiology,
University of Karachi,
Karachi-75270,
PAKISTAN.