

ROTAVIRUSES IN INFANTS WITH DIARRHEA STUDIED BY VIRAL RNA ELECTROPHORESIS IN ANKARA, TURKEY*

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Rotavirus gastroenteritis is one of the major causes of mortality in children in developing countries, with an estimated 500,000 deaths annually¹. In developed countries rotaviruses are clearly the most common causative agents in childhood diarrhea², whereas in many developing countries they are overshadowed by the incidence of bacterial diarrheas³⁻⁹. However, in view of the potential prevention of rotavirus diarrhea by vaccination^{10,11} it is important to establish the etiologic role of rotaviruses in any given area in the world. The present communication is the first study of rotavirus epidemiology in Turkey where infantile gastroenteritis is a significant problem.

This study was carried out using viral RNA electrophoresis in agarose gels as a diagnostic method for rotavirus¹²⁻¹⁹. The diagnosis is based on direct identification of typical rotavirus RNA segments from stools. The method appears specific, simple and economic, making it suitable for application in developing countries^{15,16,18}.

Material and Methods

During a one-year period, from July 1984 through June 1985, stool specimens were collected from 375 children under two years of age admitted for acute diarrhea to Hacettepe Children's Hospital and Dr. Sami Ulus Children's Hospital in Ankara. All the patients in the diarrhea group presented with an acute onset of

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diarrhea which was less than four days duration. The stool specimens were obtained from each patient upon admission.

The group of 375 children selected were a random sampling among the total 14,370 admissions for diarrhea to the two hospitals during the study period. At the same time, throughout the study period, stool specimens were collected from a control group of 333 children without diarrheal disease.

The viral RNA electrophoresis in agarose gels was performed according to the method of Espejo et al¹⁴. Specimens which exhibited the first four of the nine characteristic rotavirus RNA segment migration bands were considered positive. Bacteriologic cultures for Salmonella, Shigella and Campylobacter were performed using standard techniques.

Results

Rotaviruses were found in 61 of the 375 children (16.3%) with diarrhea using the agarose gel RNA electrophoresis from stool specimens. In contrast, rotavirus was not detected in any of the 333 stool specimens of the control children. Salmonella, Shigella and Campylobacter were detected in a total of 51 (13.6%) of the patients with diarrhea and in 6 (1.8%) of the control children (Table I). There was one dual infection with rotavirus and Salmonella.

TABLE I: Detection of Rotavirus and Enteropathogenic Bacteria in Infants with Acute Diarrhea During a One-Year Period in Ankara, Turkey

Agent	Control group (n = 333)	Diarrhea group* (n = 375)
Rotavirus	0 (0%)	61 (16.3%)
Salmonella	1 (0.3%)	28 (7.5%)
Shigella	3 (0.9%)	16 (4.3%)
Campylobacter jejuni	2 (0.6%)	7 (2.6%)

* One case with both agents isolated (rotavirus and salmonella).

The total incidence of diarrhea was highest in the months of June, July and August, but rotavirus was uncommon in this period (Fig.1). In contrast, the highest incidence of rotavirus occurred from November to March, when the total incidence of diarrhea was lowest. In the peak month of December, rotavirus was found in 11 of 19 (58%) stool specimens studied. Rotavirus was not detected in any of the 99 stool specimens collected in July and August.

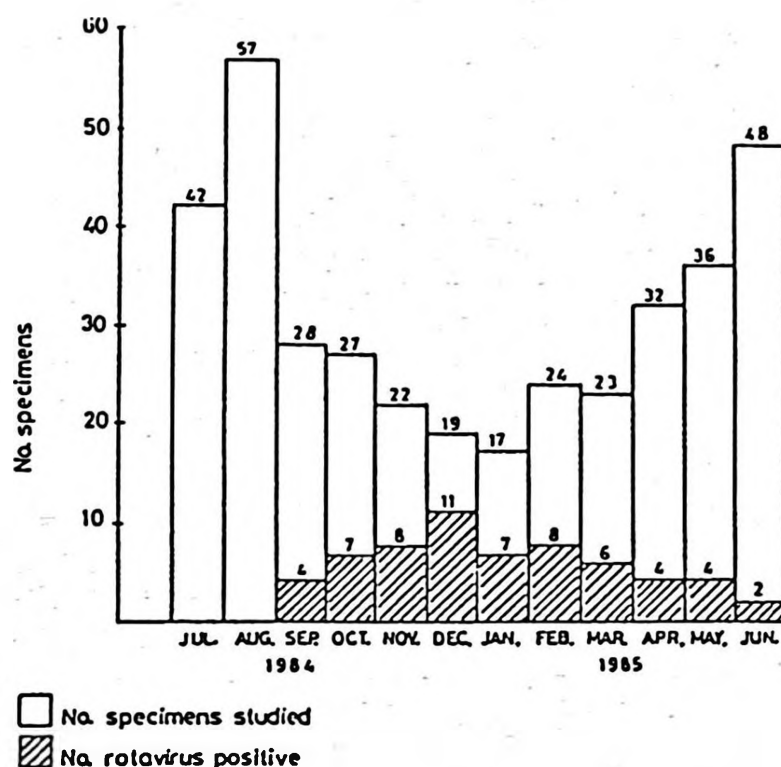


Fig 1: Monthly distribution of rotavirus in infants with acute diarrhea in a 12-month survey conducted in Ankara, Turkey. Also shown is the total number of children with diarrhea studied each month.

Discussion

In accordance with previous reports, we demonstrated by agarose gel electrophoresis, RNA pattern characteristic of rotavirus suitable for studies of rotavirus in developing countries^{15,16,18}. The sensitivity of the RNA electrophoresis method appears such that it detects primarily cases with clinical rotavirus diarrhea. Children with subclinical infection and presumably more limited virus multiplication may not excrete a sufficient amount of virus to give a positive test. This would explain the completely negative findings in our control group, which also concurs with previous reports^{15,18}.

Altogether, the present survey with the RNA electrophoresis method allowed us to estimate the role of rotaviruses in the etiology of acute diarrhea in children under two years of age in Ankara. The present study indicated that rotaviruses show a similar seasonal epidemiologic pattern in Turkey as in countries with a temperate climate², with peak incidence in cold months. This was in fact a mirror image of the occurrence of non-rotavirus (presumably bacterial) gastroenteritis, which was most common in the months of June to August. A similar "mirror in age" pattern was also seen in a previous epidemiologic survey in Finland²⁰, although the role of bacterial diarrhea there is much smaller today.

While the overall incidence of rotavirus in children with acute diarrhea found in this study was only 16%, the absolute number of hospital admissions due to rotavirus in Ankara is still quite high. On the basis of the present figures, it can be estimated that there are about 2,340 hospital admissions annually for rotavirus diarrhea in Ankara (pop. 2,700,000) alone. This figure would certainly justify attempts for prevention of rotavirus diarrhea by vaccination in the community, if an effective rotavirus vaccine were available. The seasonal distribution of rotavirus found in the present study might have implications for planning rotavirus vaccination trials in Turkey. In Finland, rotavirus diarrhea in winter has been successfully prevented by oral vaccination with the RIT 4237 strain of bovine rotavirus of infants at the age of 6 to 12 months in the fall before the epidemic season^{10,11}. In view of the present findings, such an experimental vaccination program in Turkey could be best performed in July and August.

On the other hand, it seems clear that rotaviruses account for only a minor part of total infantile gastroenteritis in Turkey. The non-rotavirus diarrhea, presumably of bacterial origin, can only be brought under control by improved hygienic conditions. Experience from Finland over the past 30 years suggests that such development will decrease the incidence of total infantile diarrhea, whereas rotavirus diarrhea is not likely to decrease; on the contrary, its proportional role will increase²¹. This emphasizes the need for specific measures to control rotavirus diarrhea.

Summary

The viral RNA electrophoresis method was used to detect rotavirus in children under two years of age, admitted to two children's hospitals in Ankara, Turkey, during the period from July 1984 through June 1985. Rotavirus was detected in 61 out of 375 children with diarrhea (16.3%), but in none of the 333 control children. Peak incidence of diarrhea occurred in December when the total incidence of diarrhea was the highest. The seasonal occurrence of rotavirus diarrhea in Turkey may have implications for planning of rotavirus vaccination. The viral RNA electrophoresis method has the advantages of simplicity, low cost, and specificity for studies of diarrhea in developing countries.

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REFERENCES

1. Walsh JA, Warren KS. Selective primary health care: an interim strategy for disease control in developing countries. *N Engl J Med* 301:967, 1979.
2. Barnett B. Viral gastroenteritis. *Med Clin North Am* 67:1031, 1983.
3. Black RE, Merson MH, Rahman AS, et al. A two-year study of bacterial, viral, and parasitic agents associated with diarrhea in rural Bangladesh. *J Infect Dis* 142:660, 1980.
4. Echeverria P, Blacklow NR, Vollet JL 3d, et al. Reovirus-like agent and enterotoxigenic *Escherichia coli* infections in pediatric diarrhea in the Philippines. *J Infect Dis* 138:326, 1978.
5. Engleberg NC, Holburt EN, Barrett TJ, et al. Epidemiology of diarrhea due to rotavirus on an Indian reservation: risk factors in the home environment. *J Infect Dis* 145:894, 1982.
6. Jonas A, Yahav J, Katznelson D, et al. Etiology of acute gastroenteritis in children in Israel: role of human reovirus-like agent and bacterial pathogens. *Isr J Med Sci* 15:754, 1979.
7. Maiya PP, Pereira SM, Mathan M, et al. Aetiology of acute gastroenteritis in infancy and early childhood in southern India. *Arch Dis Child* 52:482, 1977.
8. Pickering LK, Evans DJ Jr, Muñoz O, et al. Prospective study of enteropathogens in children with diarrhea in Houston and Mexico. *J Pediatr* 93:383, 1978.
9. Wyatt RG, Yolken RH, Urrutia JJ, et al. Diarrhea associated with rotavirus in rural Guatemala: a longitudinal study of 24 infants and young children. *Am J Trop Med Hyg* 28:325, 1979.
10. Vesikari T, Isolauri E, D'Hondt E, et al. Protection of infants against rotavirus diarrhoea by RIT 4237 attenuated bovine rotavirus strain vaccine. *Lancet* 1:977, 1984.
11. Vesikari T, Isolauri E, Delem A, et al. Clinical efficacy of the RIT 4237 live attenuated bovine rotavirus vaccine in infants vaccinated before a rotavirus epidemic. *J Pediatr* 107:189, 1985.
12. Kalica AR, Garon CF, Wyatt RG, et al. Differentiation of human and calf reovirus-like agents associated with diarrhea using polyacrylamide gel electrophoresis of RNA. *Virology* 74:86, 1976.
13. Espejo RT, Calderón E, Gonzáles N. Distinct reovirus-like agents associated with acute infantile gastroenteritis. *J Clin Microbiol* 6:502, 1977.
14. Espejo RT, Romero P, Calderon E, Gonzales N. Diagnostico de rotavirus por electrophoresis del RNA viral. *Bol Med Hosp Infant Mex* 35:323, 1978.
15. Herring AJ, Inglis NF, Ojeh CK, et al. Rapid diagnosis of rotavirus infection by direct detection of viral nucleic acid in silver-stained polyacrylamide gels. *J Clin Microbiol* 16:473, 1982.
16. Albert MJ, Bishop RF, Shann FA. Epidemiology of rotavirus diarrhea in the Highlands of Papua, New Guinea, in 1979, as revealed by electrophoresis of genome RNA. *J Clin Microbiol* 17:162, 1983.
17. Alvarez-Muñoz MT, Guiscafré-Gallardo JP, Mondragon-Sánchez C, et al. Comparison between viral RNA electrophoresis, ELISA and complement fixation techniques with electronic microscopy to demonstrate rotavirus. *Arch Invest Med* 13:145, 1982.
18. Avendaño LF, Calderón A, Macaya J, et al. Rotavirus viral RNA electrophoresis in hospitalized infants with diarrhea in Santiago, Chile. *Pediatr Res* 16:329, 1982.
19. Avendaño LF, Dubinovsky S, James HD Jr. Comparison of viral RNA electrophoresis and indirect ELISA methods in the diagnosis of human rotavirus infection. *Bull Pan Am Health Organ* 18:245, 1984.
20. Vesikari T, Mäki M, Sarkkinen HK, et al. Rotavirus, adenovirus, and non-viral enteropathogens in diarrhoea. *Arch Dis Child* 56:564, 1981.
21. Vesikari T, Mäki M, Isolauri E. Epidemiologic background for the need of rotavirus vaccine in Finland. Preliminary experience of RIT 4237 strain of live attenuated rotavirus vaccine in adults. *Dev Biol Stand (Basel)* 53:229, 1983.