Gastroenteritis in Children: A Two-Year Review in Manitoba.

I. Etiology

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During two years, 1,217 children hospitalized with gastroenteritis at the Children's Centre in Winnipeg, Manitoba, Canada were studied. Bacterial pathogens were present in 25% of these children: enteropathogenic *Escherichia coli* in 120, *Shigella* in 139, *Salmonella* in 24, and multiple pathogens in 18. Rotavirus was detected in 54 (11%) of 472 patients examined. Rotavirus and enteropathogenic *E. coli* were the most common pathogens in infants, and *Shigella* was the most common in older children. Bacterial diarrhea occurred more commonly in summer, whereas rotavirus infection occurred more commonly in winter. Among 276 children screened, enterotoxigenic *E. coli* was found in three, and *Aeromonas shigelloides* that produced a similar toxin in two others. Enteroinvasive *E. coli* was not detected in 70 children. Organisms producing toxins "cytotoxic" to HeLa cells were isolated from three of 90 children. Screening for enterotoxigenic or enteroinvasive organisms was not productive of a significant number of pathogens, and, although screening for rotavirus did improve the number of etiologic diagnoses, the etiology of the majority of cases of diarrhea remained unknown.

Despite a decline in the rate of mortality due to gastroenteritis in North America, this illness remains a major cause of infant morbidity. During the last few years, newer techniques, such as direct electron microscopic examination of feces and specialized assays for enterotoxigenic or enteroinvasive organisms, have become available. With use of these techniques, it has become apparent that in North America, England, and Australia, the rotavirus is a major cause of gastroenteritis in young children [1-5]. The role of enterotoxigenic and enteroinvasive *Escherichia*

coli has remained uncertain. The results of investigations of enterotoxigenic or enteroinvasive organisms have been remarkably varied: enterotoxigenic organisms have been found in 0-86% and enteroinvasive organisms in 0-30% of children with gastroenteritis [4-9].

This report reviews the etiology of gastroenteritis in children who were hospitalized for treatment of the illness during a two-year period in Manitoba, Canada. Special emphasis is given to these newer pathogens, the rotavirus and enterotoxigenic and enteroinvasive *E. coli*. Additional epidemiologic and clinical features of these patients are described in another report.¹

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Materials and Methods

Patients. All patients admitted with gastroenteritis to the Children's Centre, Winnipeg, Manitoba, from December 1, 1973, to November 30, 1975, were studied. The Children's Centre is a major referral hospital for children (younger than 16 years) in the Province of Manitoba and accounts for ~15% of all pediatric ad-

¹ M. J. Gurwith and D. Hinde, "Clinical Features of Gastroenteritis in Children, A Two-Year Review in Manitoba," manuscript in preparation.

missions in Manitoba, including a great proportion of referrals from the rural North. During the period reviewed, 1,217 cases of gastroenteritis admitted to the Children's Centre were identified; almost all of these patients were admitted directly to the Isolation Unit at the Children's Centre. A few children were admitted to other areas and then developed symptoms of gastroenteritis or were admitted directly to the Intensive Care Unit.

Types of investigation. From each child with the diagnosis of gastroenteritis, one to three fecal specimens or rectal swabs were sent to the clinical microbiology laboratory. Those children from whom no established bacterial pathogen (enteropathogenic *E. coli*, *Shigella*, *Salmonella*, *Yersinia enterocolitica*, or *Vibrio parahaemolyticus*) was isolated were considered to have nonbacterial gastroenteritis (NBG). Examination of fecal specimens by electron microscopy for rotavirus was commenced on a tentative basis in 1974. During 1975, we attempted to examine a fecal specimen from every child admitted with gastroenteritis for the presence of rotavirus. Fecal specimens were not available from some patients for examination, generally because the diarrhea or stay at the hospital was so brief that a specimen was not saved specifically for electron microscopic examination. Many such patients were admitted only over a weekend. During the second year of the study, from December 1974 to November 1975, fecal specimens were examined by electron microscopy from approximately two-thirds (402) of the 620 patients.

During separate periods in the two years, organisms isolated from fecal specimens were screened for production of enterotoxin. In the first such period, we attempted to screen only patients with a history of five or more stools in the 24 hr preceding admission and from whom a bacterial pathogen was not isolated. There were 53 patients in this group, admitted from March to June 1974. During the next period, starting in October 1974, we attempted to screen a group of 87 consecutively admitted patients with the diagnosis of gastroenteritis, regardless of history and associated pathogens. The third group, studied from July to September 1975, was composed of patients considered eligible for an antibiotic study of gastroenteritis. The initial fecal specimen, however, was obtained from all patients in

this group before antibiotics were administered in the hospital. In the latter group, 81 patients were screened for enterotoxigenic organisms, and of the 81, 54 were screened for enteroinvasive organisms. In addition, throughout the two years tests for enterotoxigenic organisms were performed on an ad hoc basis, when requested by attending or consulting physicians.

Fecal specimens were examined for parasites directly and by formalin-ether concentration, and/or were sent to the Manitoba Provincial Virology Laboratory for viral isolation from a small and unselected group of children. Previously in Manitoba, fecal specimens from patients with gastroenteritis have had a consistently low yield of viral pathogens or pathogenic intestinal protozoa.

Bacteriologic methods. Blood agar, MacConkey's agar, hektoen agar (Baltimore Biological Laboratories, Baltimore, Md.), and selenite F broth were used for primary isolation of organisms in the fecal specimen. Enteropathogenic *E. coli* were identified with use of commercial antisera. In general, 10 separate colonies from each specimen were tested as recommended by Ewing and Martin [10] with use of Difco pools A and B (Difco Laboratories, Detroit, Mich.) and, after April 1975, pool C. Pool A consisted of serotypes O26:K60, O55:K59, O111:K58, and O127a:K63; pool B of serotypes O86a:K61, O119:K69, O124:K72, O125:K70, O126:K71, and O128:K67; and pool C of serotypes O18aO18c:K77, O20aO20c:K61, O20a-O20b:K84, O28:K73, O44:K74, and O112aO112c:K66. *Salmonella*, *Shigella*, and other aerobic gram-negative bacilli were identified from an initial fecal culture with use of standard microbiologic techniques [10].

Assay for enterotoxin production or enteroinvasiveness. In the assay for enterotoxigenic or enteroinvasive organisms, five to 10 colonies resembling *E. coli* and one isolate of any other gram-negative bacilli were picked from the original fecal culture and saved on trypticase soy agar slants. Each isolate was then inoculated into 8 ml of brain-heart infusion (BHI) broth and incubated (without shaking) for 48 hr at 37 C. Sterile supernatants from these cultures were maintained at 4 C and were tested within 10 days for the presence of enterotoxins or frozen at -70 C until tested. Heat-labile enterotoxin (LT) was assayed by the Y-1 mouse adrenal tumor cell sys-

tem [11]; >50% rounding of cells on at least three separate assays was considered a positive response.

Heat-stable enterotoxin (ST) was originally assayed from the same supernatants as were used in the assay for LT, but later, those of cultures (from 54 patients) that had been incubated in BHI broth with shaking for 24 hr were used. Supernatants for assay of ST were prepared only from *E. coli* isolates and within two or three months of primary isolation. Pooled litters of one- to four-day-old albino mice were used for assay of ST [8]. In our laboratory, an average ratio of the weight of small intestine to remaining body weight of >0.08 was considered a positive response. Positive and negative LT controls were included in each adrenal cell assay, and a positive ST control (provided by Dr. R. Bradley Sack, Johns Hopkins University Medical School, Baltimore, Md.) was included in approximately every fourth infant mouse assay. Heat lability or stability of an enterotoxin preparation was confirmed by heating at 100 C for 30 min and retesting. In assays for "cytotoxic" enterotoxins [12], only one isolate of *E. coli* and one isolate each of all of the aerobic gram-negative bacilli were tested with use of supernatants from BHI broth cultures that had been agitated for 24 hr. Assays for cytotoxic enterotoxin were performed on patients admitted from June to September 1975. HeLa cell cultures were overlayed with a 1:5 dilution of each supernatant and examined after one day for the presence of visible cytotoxicity.

Enteroinvasiveness was assayed with use of the Serény test; $\sim 10^8$ organisms were inoculated directly into the conjunctival sac of a guinea pig. Subsequent conjunctival and corneal inflammation was considered a positive response [13]. In most cases, all 10 isolates of *E. coli* from the same patient, after being grown separately in BHI broth, were pooled and inoculated together in the guinea pig conjunctival sac. Positive invasive *E. coli* controls (provided by Dr. Samuel Formal, Walter Reed Army Institute of Research, Washington, D.C.) were always positive when tested in this way, even when pooled with 10 negative organisms. A pool of 10 isolates of *E. coli* from each child was tested in one eye of two separate guinea pigs, and with each assay a posi-

tive invasive control was included in a duplicate pool of 10 organisms from one patient.

Electron microscopic examination of feces. Fecal specimens were examined by a modification of the method described by Bryden et al. [1]. The specimen was emulsified in 8 ml of filtered distilled water and centrifuged at 6,000 g. The supernatant was then placed directly onto Formvar-coated grids (Ladd Research Industries, Burlington, Vt.) and stained with 1.5% phosphotungstic acid. Rectal swabs were emulsified in a small amount of filtered distilled water, and the resultant suspension was treated as above. Each grid was examined on a Philips 201 electron microscope (Philips Electronics Ltd., Scarborough, Ontario, Canada) for a minimum of five squares on a 400-mesh grid or until rotavirus-like particles were recognized. All particles identified as viruses were confirmed by examination of a photographic enlargement.

Results

Assay for enterotoxigenic and enteroinvasive organisms. During the two-year period, organisms isolated from 276 (23%) of 1,217 children were screened in the mouse adrenal tumor cell assay for the ability to make LT or cytotoxic enterotoxin [12]. These included 1,379 separate isolates of *E. coli* and 306 isolates of other aerobic gram-negative bacilli including two of *Salmonella*, 35 of *Shigella*, three of *Aeromonas*, 49 of *Proteus*, and 139 of *Klebsiella*. These isolates were obtained from children with diarrhea during all seasons. Organisms obtained from a smaller sample of children (6% of 1,217) were screened for the ability to make ST or cytotoxic enterotoxin [12] and for enteroinvasiveness (table 1).

Only five patients were found to have organisms positive in the mouse adrenal tumor cell system. In three of these patients, *E. coli* that produced typical LT were found: *E. coli* type O?:H40, isolated in October 1974, producing only LT; *E. coli* O75:H— (H— designating nonmotile), isolated in August 1975, also producing only LT; and *E. coli* O6:H16, isolated in September 1975, producing both LT and ST. In addition, *Aeromonas shigelloides* was isolated from two patients, and both isolates were found to produce a

Table 1. Results of assays for toxigenic and enteroinvasive organisms in children hospitalized with gastroenteritis in Winnipeg, Manitoba, Canada.

Assay system	Property assayed	No. of patients	No. of isolates	No. of patients with positive isolate (species)*
Mouse adrenal tumor cells	"Cytotoxic" toxin [12]†	276	1,685	3 (<i>Escherichia coli</i>) 2 (<i>Aeromonas shigelloides</i>)
Infant suckling mouse	Heat-stable <i>E. coli</i> enterotoxin	74	204	1 (<i>E. coli</i>)
HeLa cells	"Cytotoxic" toxin [12]	90	170	1 (<i>Pseudomonas aeruginosa</i>) 1 (<i>E. coli</i>) 1 (<i>Aeromonas hydrophila</i>)
Guinea pig conjunctivae	Enteroinvasiveness	70	595	0

*One patient had both *A. shigelloides* and *A. hydrophila* in the same fecal specimen, and one had *E. coli* (O6:H16) that produced toxins positive in both the mouse adrenal tumor cell and infant suckling mouse assays.

†Cholera enterotoxin and heat-labile enterotoxin of *E. coli*, which stimulate adenylate cyclase, are routinely detected in this system.

toxin which caused rounding in the mouse adrenal tumor cells indistinguishable from that produced by *E. coli* LT, except that there was no loss of activity when the toxin was heated at 100 C for 30 min. This observation was confirmed in three separate assays by coding of the slides and counting of all the cells in 20 equidistant preselected fields on a Lab-Tek slide culture (Lab-Tek Products, Division of Miles Laboratories, Naperville, Ill.) stained with hematoxylin and eosin; a minimum of 200 cells were counted. The isolates of *A. shigelloides* produced 45%–60% rounding of cells, with and without heating, as compared with 5%–20% rounding seen with negative and heat-inactivated LT controls.

With use of the HeLa cell assay, organisms producing a cytotoxic toxin were detected in fecal cultures from three children. These organisms (*E. coli*, *Pseudomonas aeruginosa*, and *Aeromonas hydrophila*) were found to produce a cytotoxic substance that caused easily visible necrosis and death in HeLa cells. *A. hydrophila* and *P. aeruginosa* had been isolated from eight of 276 children, but only two of these isolates were available for testing. The toxin-producing *P. aeruginosa* was the only organism present in the fecal specimens from which it was isolated. No effect was produced in the HeLa cell assay by the *A. shigelloides* toxin or *E. coli* LT.

Only one isolate of *E. coli*, the same *E. coli* type O6:H16 already detected in the mouse adrenal tumor cell system, was found to be ST-positive with use of the infant suckling mouse assay. No

other ST-positive strains of *E. coli* were detected, and none of the three strains of *Aeromonas* was positive in this assay.

No enteroinvasive organisms were detected with use of the guinea pig conjunctival assay.

Electron microscopic examination of fecal specimens. Rotavirus was visualized in fecal specimens of 54 (11%) of 472 children from whom specimens were available for examination. Thirteen rectal swabs and 594 stool specimens from these 472 children were tested; most of these specimens were collected during the second year of study. Rotavirus was identified in 45 (13%) of 342 children from whom no bacterial pathogens were isolated and in nine (7%) of 130 children whose fecal cultures had a bacterial pathogen (no significant difference; $0.10 > P > 0.05$). Among patients whose fecal specimens were available for electron microscopic study, rotavirus was identified in three of 64 with isolates of enteropathogenic *E. coli* and in five of 44 with isolates of *Shigella*. In addition, there were particles morphologically resembling adenovirus in seven fecal specimens, coronavirus in one, and picornavirus or parvovirus in three.

Epidemiologic features. NBG was by far the most common etiology, diagnosed in ~80% of children under one year of age and in 67% of those three years old or older (table 2). Included as a subgroup of NBG were those children shown to be rotavirus-positive or -negative by electron microscopy. In the group of patients with NBG, a rotavirus was most common in those younger than one year, occurring in 16 (11%) of 146 who

Table 2. Detection of rotavirus by age category in children hospitalized with gastroenteritis in Winnipeg, Manitoba, Canada.

Age (months)	Non-bacterial gastro-enteritis*	Bacterial gastro-enteritis	Total†
0-5	16/146 (11)	6/57 (11)	22/203 (11)
6-11	14/85 (16)	0/27	14/112 (13)
12-35	13/87 (15)	3/38 (8)	16/125 (13)
>36	2/24 (8)	0/8	2/32 (6)
Total	45/342 (13)	9/130 (11)	54/472 (11)

NOTE. Data are given as number of patients positive for rotavirus/number of patients screened (percentage). At least one fecal specimen per patient was examined by electron microscopy.

*Differences between percentages of patients positive for rotavirus by age group were not significant ($P > 0.50$; 3 degrees of freedom; $\chi^2 = 1.80$).

†Differences between percentages of patients positive for rotavirus by age group were not significant ($P > 0.70$; 3 degrees of freedom; $\chi^2 = 1.13$).

were younger than six months and in 14 (16%) of 85 aged six to 11 months. In contrast, rotavirus was detected in only two (8%) of 24 patients older than 36 months. If those children with a bacterial pathogen whose fecal specimens were also examined for rotavirus are included, the percentages of rotavirus-positive patients are similar, ranging from 13% of those six to 35 months old to 6% of those older than 36 months. The small differences in the percentages of rota-

virus-positive children in different age groups are not statistically significant, whether or not children with bacterial infections and rotavirus are included (table 2).

Enteropathogenic *E. coli* were present in 14% and 13% of those younger than five months and those six to 11 months old, respectively, and in only 3% of children three years old or older (table 3). In contrast, *Shigella* were present in only 6% and 7% of those up to five months and those six to 11 months old, respectively, and the percentage rose to 28% for those three years old or older. Both the decrease in enteropathogenic *E. coli* with increasing age and the increase in *Shigella* with increasing age were statistically significantly different from the overall age distributions for the entire population of children with diarrhea.

Gastroenteritis associated with *Salmonella*, *Shigella*, and enteropathogenic *E. coli* occurred most commonly in the summer and fall, although cases did occur throughout the year (figure 1). In the four summer months (June through September) when rotavirus was identified only once, gastroenteritis associated with a bacterial pathogen accounted for 169 (40%) of 425 patients. In contrast to the bacterial diarrheas, NBG occurred most often in the winter months with the lowest occurrence in the spring. Rotavirus was almost exclusively a winter pathogen and was found in 25% of 160 children tested in November through

Table 3. Etiology of gastroenteritis correlated with age among children hospitalized in Winnipeg, Manitoba, Canada.

Etiologic category	Age (months)				Total
	0-5	6-11	12-35	>36	
Nonbacterial*	313 (78)	222 (80)	275 (75)	106 (67)	916 (76)
Bacterial					
Enteropathogenic <i>Escherichia coli</i> †	56 (14)	35 (13)	28 (8)	4 (3)	123 (10)
<i>Shigella</i> ‡	25 (6)	19 (7)	55 (18)	45 (28)	144 (12)
<i>Salmonella</i>	8 (2)	2 (1)	10 (3)	4 (3)	24 (2)
Total	89 (22)	56 (20)	93 (25)	53 (33)	291 (24)
Total§	402 (100)	278 (100)	368 (100)	159 (100)	1,207 (100)

NOTE. Data are given as number of patients with indicated category of gastroenteritis (percentage of total number of patients in age group with gastroenteritis).

*Includes patients tested for rotavirus.

†The decrease in enteropathogenic *E. coli* with increasing age is statistically significant ($P < 0.001$; 3 degrees of freedom; $\chi^2 = 17.3$).

‡The increase in *Shigella* with increasing age is statistically significant ($P < 0.001$; 3 degrees of freedom; $\chi^2 = 57.6$).

§Includes total for nonbacterial and bacterial gastroenteritis, and excludes 10 patients with more than one bacterial pathogen.

March. During these same winter months, a bacterial pathogen was identified in only 66 (12%) of 563 children, a statistically significant decrease from the summer rate ($P < 0.001$, 1 degree of freedom, $\chi^2 = 103.5$).

Discussion

In the past, a specific etiology has not been detected in the majority of cases of gastroenteritis in children. Although there is some variation depending on location and time of year, in most published studies a bacterial pathogen was found in only 15%–35% of patients [14–16]. The etiologic role of common viruses in gastroenteritis has remained questionable. In a review of many studies, enteroviruses and adenoviruses were found with no greater frequency among children with diarrhea than among controls [15].

Our own two-year review of gastroenteritis in hospitalized children also suggests that bacterial pathogens account for a minority of cases. Likewise, the small number of viral isolations, although based on a small sample, resembles findings in other studies [15]. The consistently low yields of viral pathogens from patients with gastroenteritis previously noted in Manitoba (J. C. Wilt, personal communication) has tended to decrease the number of specimens submitted for viral isolation. Although it is possible that intestinal protozoa could have been detected more often, these organisms are uncommon in Manitoba even when fecal specimens have been screened optimally (authors' unpublished observations).

With the recognition of the capacity of some *E. coli* to cause diarrhea by enterotoxin production or mucosal invasion, it was thought that such organisms, distinguishable as pathogenic from

Figure 1. Number of cases of gastroenteritis per month at the Children's Centre in Winnipeg, Manitoba, Canada by etiological category. Other = *Salmonella* or multiple bacterial pathogens; NBG = nonbacterial gastroenteritis (excluding children tested for rotavirus); total NBG includes all children tested for rotavirus.

other *E. coli* only by these properties, might account for a large proportion of endemic gastroenteritis [17]. Recent investigations suggest that in developing countries such organisms do account for a significant proportion of endemic diarrhea and are present in 20%–56% of native cases [18–20] and also in diarrhea of travelers to such areas [21–23]. However, even in areas where enterotoxigenic organisms are common, they are numerically more important in adults than in children [20]. In North America and Europe, enterotoxigenic or enteroinvasive organisms have not been shown to be a numerically important cause of diarrhea in children.

Only two of the many studies from North America have shown high rates of identification of these organisms in children. In an early study from Chicago, toxigenic *E. coli* were found in 24 of 29 infants with diarrhea; however, toxigenicity was assayed in an infant rabbit model, a system not used in most other published studies [6]. In a study from Dallas, Texas, *E. coli* that produced ST were found in 86% and enteroinvasive *E. coli* in 30% of children with diarrhea, whereas ST-producing organisms were found in 41% and enteroinvasive organisms in 12% of controls [7]. These high rates of enterotoxigenic or enteroinvasive *E. coli* have not been reported elsewhere in either patients with diarrhea or controls.

All other published investigations of diarrhea in children in North America showed a low incidence of enterotoxigenic *E. coli* and, when studied, an absence of enteroinvasive *E. coli*. Enterotoxigenic *E. coli* were found in only 16% of 64 children with diarrhea on an Indian reservation in Arizona [9]. None of 130 children with diarrhea investigated in Washington, D.C., Boston, and Honolulu were shown to have enterotoxigenic *E. coli* [4, 5, 8]. Our results suggest that enterotoxigenic and enteroinvasive *E. coli* are uncommon in children: enterotoxigenic organisms occurred in <2% of those investigated, and no enteroinvasive organisms were detected. Enteropathogenic *E. coli* isolated during the study from hospitalized children were also negative when assayed for these properties [24], a finding which reflects that of two other investigations [25, 26].

Enterotoxins other than cholera toxin and *E.*

coli LT and ST have been previously recognized, although none of these, with the possible exception of that associated with *Shigella dysenteriae*, has been as well studied. Enterotoxins have been associated with a variety of organisms, including *Clostridium perfringens*, *S. dysenteriae*, *P. aeruginosa*, *Klebsiella* species, and *Enterobacter* species [27, 28]. The enterotoxins associated with *C. perfringens* and *S. dysenteriae* have been called "cytotoxic," in contrast to cholera and *E. coli* enterotoxins, which have been called "cytotoxic" [12]. Cytotoxic toxins stimulate steroid secretion in tissue culture systems rather than cause cell death as is seen with cytotoxic toxins. Screening for organisms that produce cytotoxic toxins has not been a feature of other published studies of diarrhea in children. Some cytotoxins would not be detected in the mouse adrenal tumor cell system, but would require HeLa cells for their identification [12]. The identification of such a toxin produced by an isolate of *A. hydrophila* from an adult with severe diarrhea [29] and a similar finding by others [30] prompted us to screen for production of cytotoxic toxin in a sample of aerobic enteric gram-negative bacilli already saved from our study. In this limited sample, only three cytotoxic toxin-producing organisms were detected. It does not appear that such screening will identify an important etiologic group. The clinical significance of the identification of organisms producing cytotoxic toxins also remains to be defined.

In the mouse adrenal tumor cell assay, we found only two isolates of organisms other than *E. coli* that produced changes resembling those produced by *E. coli* LT. Both isolates were *A. shigelloides*. The substance produced by *A. shigelloides* differs from *E. coli* LT in being heat-stable. *A. shigelloides* has been associated with diarrhea [31], but further characterization of the toxin produced by this organism will be required to determine whether it has any clinical significance. Although enterotoxins resembling the cholera toxin and *E. coli* LT but produced by other organisms have been reported by others [18, 32], these toxins have not yet been adequately characterized.

The importance of the rotavirus in gastroenteritis of children is increasingly evident. In several large investigations from diverse localities, this pathogen has been found to account for

38%–50% of the cases of diarrhea in children and has been present in up to 70% of children during the winter months [1, 2, 4]. In Manitoba, we also found that the rotavirus was numerically an important pathogen, although found at a lower rate (11% overall and 25% during the winter months) than that reported elsewhere. Our lower rate may reflect the fact that we attempted to look for rotavirus in all children admitted with diarrhea in 1975. Rotavirus was uncommon in children older than 36 months, and the highest rate was in those six to 11 months old. However, since diarrhea leading to hospitalization was most common in children younger than six months, rotavirus was found most frequently in this age group. A winter peak as well as the near complete absence of rotavirus during the summer have also been reported [1, 2, 4], although no explanation for these findings is apparent.

In conclusion, in Manitoba bacterial pathogens were present in 25% of children hospitalized with gastroenteritis; enteropathogenic *E. coli* were more common in infants, and *Shigella* were more common in older children. Rotavirus was numerically an equally important pathogen and was present in up to 25% of children with gastroenteritis during the winter. Although a small number of enterotoxigenic organisms were detected, it does not appear that enterotoxigenic organisms were of great numerical importance in the etiology of diarrhea in hospitalized children or that any sort of routine screening, at least with use of the four assay systems (mouse adrenal tumor cells, infant suckling mice, HeLa cells, or guinea pig conjunctival inoculation), will be productive. It also appears that the etiology of the majority of cases of diarrhea in children remains unknown.

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