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Clinical features of acute gastroenteritis associated with human reovirus-like agent in infants and young children

Between January, 1974, and June, 1975, infection with a human reovirus-like agent was detected in 47% of 152 infants and children hospitalized with acute gastroenteritis. Certain epidemiologic, clinical, and laboratory findings appear to be helpful in distinguishing gastroenteritis due to HRVLA from other causes in those children sick enough to require hospitalization. Age: 76% of infants and children seven through 12 months of age and 76% of those 13 through 24 months of age had infection with the HRVLA, whereas such infection was found in only 21% of infants under six months of age and 23% of children 25 through 60 months of age. Time of Year: 61% of patients studied during the cooler months had HRVLA infection and such infection was not found from June to October. Frequency of vomiting and dehydration: Twice as many patients infected with HRVLA as those who were not had vomiting (92%) and significant dehydration (83%).

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IN 1973 BISHOP AND ASSOCIATES[1] observed virus particles with a diameter of 70 nm by electron microscopy of epithelial cells from the duodenal mucosa of children with acute nonbacterial gastroenteritis. Since then a similarly sized reovirus-like agent has been described from various parts of the world as an important, possibly the most important, etiologic agent in acute gastroenteritis of infants and young children.[2-8]

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The agent has variously been called orbivirus, duovirus, rotavirus, infantile gastroenteritis virus, and human reovirus-like agent, the term which we have been using pending resolution of nomenclature. In 1974 and 1975 we found evidence of infection with this agent in almost half of 143 infants and children hospitalized with gastroenteritis.[8] The purpose of this paper is to describe the salient clinical features in this group of infants and children who

Abbreviations used

HRVLA:	human reovirus-like agent
IGV:	infantile gastroenteritis virus
EM:	electron microscopy
IEM:	immune electron microscopy
SS:	*Shigella, Salmonella* agar
CBC:	complete blood count
BUN:	blood urea nitrogen
NCDV:	Nebraska calf diarrhea virus
EPEC:	enteropathogenic *Escherichia coli*

were ill enough with HRVLA infection to require hospitalization.

MATERIALS AND METHODS

Infants and children hospitalized with acute gastroenteritis at Children's Hospital National Medical Center were screened on admission and selected for study if they had diarrhea for a period of four days or less prior to admission. From January through December, 1974, infants and children predominantly through 24 months of age (87 patients total) were admitted to the study. Later, all patients hospitalized with acute gastroenteritis, regardless of age, were included. Informed consent was obtained from the parent or guardian in each case.

Shortly after admission, certain specimens were obtained from each patient with acute gastroenteritis and processed as follows: (1) A nose and throat swab and an anal swab were inoculated into human embryonic kidney, HEp-2, and rhesus monkey kidney cells for virus isolation; the throat swab was additionally inoculated into WI-38 cells and a diphasic mycoplasma medium. (2) Anal swabs were plated at the bedside into eosin methylene blue agar, *Shigella-Salmonella* agar, phenyl ethyl alcohol agar, and gram-negative broth for isolation of enteric bacterial pathogens. (3) Up to four diarrheal stools or occasionally anal swab specimens were examined by electron microscopy and/or immune electron microscopy. (4) Blood samples were drawn for serologic tests. The study group of 152 included nine patients from whom a stool or rectal swab specimen was not obtained but from whom paired sera were available.

The clinical records of 150 of these patients were examined retrospectively by three of us. Two records were unavailable for analysis. The patients were grouped into four categories of dehydration: none, mild, moderate, or severe.

Initial clinical laboratory studies included complete blood count, blood urea nitrogen, serum electrolytes, urinalysis, and urine specific gravity. Whenever possible, these studies were repeated until test results returned to normal. Bacterial cultures of blood, urine, and spinal fluid were made when indicated by the patient's clinical condition. Mucoid portions of diarrhea stools from 72 patients were smeared and air dried on glass slides. These slides were Wright stained and examined for the presence of leukocytes or leukocyte fragments.

At least one stool and/or swab specimen from each child hospitalized with gastroenteritis was examined by EM or IEM as described.[9, 10] For EM examination, stools were prepared into 2% filtrates and examined routinely by using an homologous convalescent serum or immune human serum globulin as the source of antibody.[6, 11, 12]

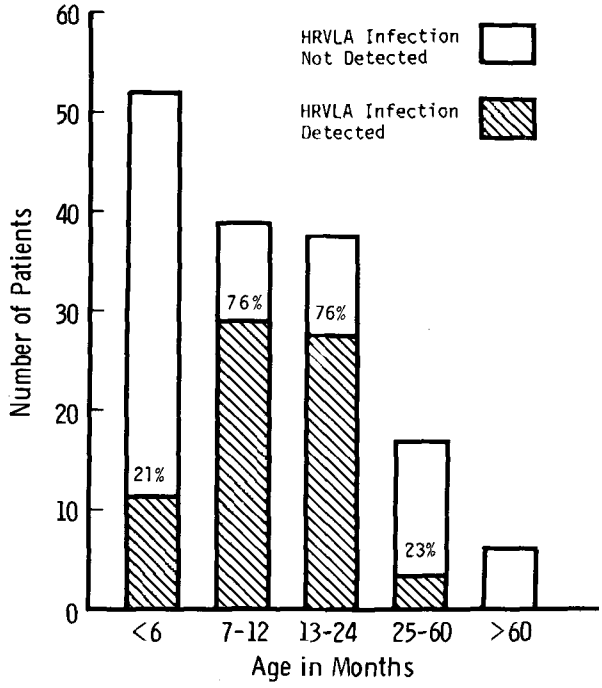

Fig. 1. HRVLA infection in relation to age in 152 patients hospitalized with gastroenteritis.

Paired acute and convalescent sera from 117 of the 152 patients studied were tested for complement-fixing antibody response to HRVLA stool filtrates antigen and/or Nebraska calf diarrhea virus, a closely related agent.[6, 8, 13-15]

Typing of enteric pathogenic bacteria was according to routine methods. Polyvalent sera for enteropathogenic *Escherichia coli* were available only for groups A and B. Typing of EPEC was carried out routinely only on specimens obtained from patients under 24 months of age. Heat labile enterotoxin production of a selected number of *E. coli* strains was searched for as described earlier.[8]

RESULTS

Seventy-two (47%) of 152 patients with gastroenteritis had evidence of infection with HRVLA (Table I). This was documented by visualization of the 70 nm agent in 42% of 143 children from whom a stool or rectal swab was available and by demonstration of a fourfold or greater rise in complement fixing antibody to the HRVLA and/or NCDV in 44% of 117 children with appropriate sera. Sixty-one percent of 118 children studied from the months of November through May were infected whereas none of 34 studied from June through October had evidence of infection. Only 8% of 76 control patients studied during the two study periods were found to be infected; one of

Table I. Evidence of infection with human reovirus-like agent (HRVLA) in hospitalized patients with gastroenteritis during periods of high and low prevalence of such infection January, 1974, to June, 1975

	Evidence of infection with HRVLA					
	HRVLA visualized by EM		*4-fold or > rise in CF antibody to HRVLA and/or NCDV*		*Total*	
Study period	*No. tested*	*No. positive (%)*	*No. tested*	*No. Positive (%)*	*No. tested*	*No. positive (%)*
HRVLA prevalent*	110	60 (54.5)	94	51 (54.3)	118	72 (61.0)
HRVLA not prevalent†	33	0 (—)	23	0 (—)	34	0 (—)
Total	143	60 (42.0)	117	51 (43.6)	152	72 (47.4)

*Jan. 74 to May 74 and Nov. 74 to May 75.
†June 74 to Oct. 74 and June 75.

these, who also shed the HRVLA, had diarrhea at the time of admission.

Among the patients with gastroenteritis, 13% of the 72 who had infection with HRVLA also had some other potential intestinal pathogen, whereas 55% of the 80 who did not have HRVLA infection had a potential intestinal pathogen (Table II). None of 275 stool bacterial isolates from 29 patients admitted with diarrhea during the cooler months (November through April) and from three patients admitted during the warmer months produced heat labile toxin.[8] The greater frequency of *Shigella* and *Salmonella* isolates in the children with no HRVLA infection is probably indicative of a true etiologic role for these agents. Since we were not studying anal swabs from hospitalized controls by conventional virologic techniques, we cannot say what the incidence of adenovirus or enterovirus recovery would have been in controls. However, serum samples were available from controls and we compared the serologic response to an adenovirus type 2 group CF antigen in paired sera of controls with those of the study patients. Three (3.6%) of 83 patients with gastroenteritis and two (2.7%) of 75 control patients had a fourfold or greater rise in adenovirus CF antibody.

With respect to bacteria from the nose and throat, over 65% of samples from both diarrhea groups were reported as containing "normal flora" and there was no unusual pattern of bacteria in either group.

CLINICAL PATTERNS

One hundred and fifty-two infants and children aged one through 155 months were included in the study (Fig. 1). Seventy-six percent of infants and children seven through 12 months of age and 76% of those 13 through 24 months of age had infection with the HRVLA, whereas such infection was found in only 21% of infants under six months of age and 23% of children 25 through 60 months of age. Furthermore, 79% of the patients with HRVLA infection were seven through 24 months of age. The two youngest patients shedding HRVLA were each 35 days of age.

There was no significant difference in the male-to-female patient ratios (HRVLA vs non-HRVLA, 1.2:1 vs 1.6:1), mean peak fever (HRVLA vs non-HRVLA, 39.5°C vs 39.4°C), or history of concurrent upper respiratory infection, (HRVLA vs non-HRVLA, 29% vs 21%). The incidence of reported gastroenteritis in relatives was 13% for the HRVLA-infected group and 10% for the non-HRVLA-infected group.

A higher proportion of HRVLA patients than non-HRVLA patients vomited (Table III). The frequency of vomiting episodes in the HRVLA group was also greater (HRVLA mean nine times, range two to 20 times/day; non-HRVLA mean three times, range one to 13 times/day). Interestingly only 72% of HRVLA-infected infants under six months of age had vomiting, as contrasted with 98% of older infants. Vomiting preceded diarrhea in the majority of patients with HRVLA infection.

PHYSICAL FINDINGS

Eighty-three percent of the patients with HRVLA infection had dehydration (Table III) as compared to 40% in the non-HRVLA group. When dehydration was diagnosed it was mostly mild to moderate for both groups. Ninety-five percent of the dehydration in the HRVLA group was isotonic as compared with 77% in the other

Table II. Occurrence of potential pathogens in 152 patients hospitalized with acute gastroenteritis

Potential pathogen	No. having other potential pathogen	
	HRVLA infection detected (72 patients)	HRVLA infection not detected (80 patients)
Shigella spp.	1	8
Salmonella spp.	2	5
Enteropathogenic *E. coli*	4	6
Adenovirus	1	15
Enterovirus	1	10
Total	9 (13%)	44 (55%)

patients with gastroenteritis. There was no difference in irritability or lethargy between the two groups. Patients with HRVLA infection as compared to their non-HRVLA counterparts were somewhat more likely to have erythema of the pharynx, red tympanic membranes, or lymphadenitis.

CLINICAL COURSE

Vomiting not only occurred more often in the HRVLA-infected patients than in the others with gastroenteritis but it lasted longer—a mean of 2.6 days as compared with 0.9 days. Diarrhea started later but lasted longer than vomiting (a mean of five days as compared with 2.6 days) in the HRVLA-infected patients, and fever in those who had it lasted an average of three days. The duration of diarrhea and fever was essentially the same in patients who had HRVLA infection and those who did not.

Once patients were hospitalized, the duration of symptoms for both groups was about the same: vomiting, approximately four hours for both groups; fever of 37.9°C or above, 1.3 days for HRVLA, and 1.6 days for non-HRVLA. Once hospitalized, diarrhea lasted a mean of 2.6 days for the HRVLA group (range 1 to 9 days) and 3.8 days mean (range 1 to 16 days) for the non-HRVLA counterparts.

Gastroenteritis was managed essentially by restriction of oral fluids and administration of parenteral fluids. Oral fluid restriction was continued for a mean of 24 hours for the HRVLA groups vs a mean of 16.5 hours for the non-HRVLA group. The mean duration of intravenous fluid therapy was essentially the same in both groups, about two days.

Patients in the HRVLA group required a mean of four days' hospitalization (range two to 14 days), whereas

Table III. Clinical characteristics of 150 children hospitalized with acute gastroenteritis

Clinical finding	Percent having each clinical finding	
	HRVLA infection detected (72 patients)	HRVLA infection not detected (78 patients)
Vomiting	96*	58*
Fever (C)		
37.9-39	46	29
> 39	31	33
Total	77	61
Dehydration†	83†	40†
Hypertonic	5	16
Isotonic	95	77
Hypotonic	0	6
Irritability	47	40
Lethargy	36	27
Pharyngeal erythema	49	32
Tonsillar exudate	3	3
Rhinitis	26	22
Red tympanic membrane with loss of landmarks	19	9
Rhonchi or wheezing	8	8
Palpable cervical lymph nodes	18	9

*Two clinical records unavailable; accordingly two subjects are excluded.
†P = 0.01.
‡P = 0.01.

patients in the non-HRVLA group were hospitalized for a mean of six days (range two to 27 days). Recovery was uneventful for patients with HRVLA infection.

CLINICAL LABORATORY FINDINGS

On admission, patients with HRVLA infection were twice as likely as their counterparts to have a blood urea nitrogen level above 18 mg/dl (58% vs 24%, $p < 0.01$) and urine specific gravity elevations over 1.025 (71% vs 33%, $p < 0.01$). Urine specific gravity values of patients with HRVLA infection returned to normal more slowly than did those in the non-HRVLA group. There were no significant differences between the two groups with respect to protein in the urine. Acetonuria paralleled observations for dehydration, BUN, and specific gravity (HRVLA 90% vs non-HRVLA 50%). The degree of acidosis and electrolyte imbalance was comparable in the two groups.

All of the cerebrospinal fluid cultures (30 HRVLA, 53 non-HRVLA) and blood cultures (48 HRVLA, 60 non-HRVLA) were negative for bacterial pathogens. Positive

urine cultures for *E. coli* greater than 100,000 colonies per milliliter were reported for 4% of the HRVLA group and 11% of the non-HRVLA group, respectively. Peripheral white blood cell counts were found mostly within a range of seven to 12 × 10^3/mm^3 for both groups on admission and thereafter.

None of the 11 chest radiographs taken from patients with HRVLA gastroenteritis infection was classified as abnormal. Fecal material from 72 patients was examined for an excess of leukocytes. Excess leukocytes were found in six of 38 (16%) who had HRVLA infection; only one of these also had a bacterial pathogen. Excess fecal polymorphonuclear cells were found in six of 34 (18%) who did not have proven HRVLA infection; four of these had a bacterial pathogen. In the total group excess fecal leukocytes were found in all five who had at least one bacterial pathogen and 7 of 67 who did not.

DISCUSSION

The clinical and epidemiologic pattern described above for patients with HRVLA infection corresponds very closely with what Zahorsky in 1929 called "winter vomiting disease."[17] Even without EM or other improved viral diagnostic methods, a winter occurrence, a young age, a high frequency and early onset of vomiting, and rapid dehydration should strongly suggest HRVLA infection to the clinician.

The pathophysiologic role of the HRVLA in inducing vomiting remains to be elucidated. Although the virus was visualized within duodenal epithelium by early investigators,[1] there has been no report of its association with gastritis. However, we are using the term gastroenteritis in keeping with the custom of other investigators.[4, 16]

In our study the fecal leukocyte test was sensitive in documenting the presence of potential enteric bacterial pathogens; the test failed in its specificity since it was also positive in the stools of seven patients (10%) in whom no potential enteric bacterial pathogens could be demonstrated, including five with HRVLA infection.

With our methods of detection, we failed to identify an etiologic agent in 45% of the non-HRVLA group (20% of the 152 patients). Etiologic possibilities include (1) enterotoxins not detected by our methods and (2) enteric viruses not yet identified. The relatively low frequency of enterotoxigenic *E. coli* observed by us is not without precedence among urban dwellers in the United States.[5] In this study, 27 nm size particles were visualized infrequently.[8]

The patients reported here were all sufficiently ill to be hospitalized but HRVLA infection apparently has a much broader clinical spectrum. For example, from December, 1975, to February, 1976, we found HRVLA in 46% of anal swab samples from 46 outpatients with gastroenteritis. This is probably an underestimate of the true incidence of HRVLA infection in these infants and children with less serious gastroenteritis because anal swab samples provide a smaller amount of fecal material for demonstrating HRVLA particles than do stools. Infection has also been noted in adults; 35% of 40 parents of patients infected with HRVLA studied between September, 1974, and June, 1975, were found to be infected; only three of these 14 infected contacts were symptomatic.[8, 18]

The smooth hospital course in the present group of HRVLA-infected is reassuring but others in major metropolitan centers have reported severe dehydration and even death in a small number of patients.[4, 19] The morbidity and mortality in underdeveloped countries and areas with poor access to pediatric care could be very high. A vaccine for infants would be welcome.

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