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A microbiological investigation of acute summer gastroenteritis in Black South African infants

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SUMMARY

A microbiological investigation of Black infants suffering from severe acute summer gastroenteritis revealed enteropathogenic agents in 30 out of 37 patients (81 %). Enterotoxigenic bacteria were isolated from 15 patients (41 %). A total of 16 enterotoxigenic strains were isolated, comprising 9 enterotoxigenic *Escherichia coli* strains secreting labile and stable toxin on their own and in combination, and labile-toxin secreting strains of *Klebsiella pneumoniae* (4), *Enterobacter cloacae* (2) and *Proteus vulgaris* (1). In the case of the latter three species, however, 6 out of the 7 strains were isolated from patients who were excreting other enteric pathogens, whereas only 2 out of 9 enterotoxigenic *E. coli* patients had concomitant infections with other pathogens. No invasive bacteria were isolated except for 2 shigella strains. Salmonella and shigella strains were found in four patients. No correlation was found between the enteropathogenicity of *E. coli* and its serotype. Rotavirus was observed by negative staining electron microscopy in only two patients (6 %) but using a reverse complement fixation test rotavirus antigen was detected in the stool of 17 out of 35 patients (49 %). The low EM detection rate may well be due to the patients being admitted for treatment late in the course of their illness when the degree of viral shedding has decreased below EM detectability. No significant difference in clinical presentation was noted between the various aetiological agents. Only one patient was being solely breast-fed compared to 16 % of control non-diarrhoeic infants. Evidence of malnutrition was noted in over half of our patients.

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INTRODUCTION

Acute gastroenteritis is the major cause of death in the infant population of the Black, Coloured and Asiatic communities of South Africa, and ranks second to pneumonia in the White community (Department of Statistics, Pretoria, 1974; Spencer & Coster, 1969). Despite its importance as a leading cause of morbidity and mortality, especially in developing countries, the classical enteropathogenic bacteria have been isolated in only 20–40 % of cases. Thus in the majority of cases of gastroenteritis no recognized enteric pathogen could be demonstrated until comparatively recently. Over the last 5 years the elucidation of the pathogenic mechanisms involved in infectious diarrhoea, and especially the important aetiological role of rotavirus (Lancet, 1975) and the enterotoxigenic bacteria (Sack, 1975), have resulted in a considerable reduction in the number of cases of unknown aetiology.

As yet, techniques for detecting rotavirus and enterotoxigenic bacteria have been limited to research laboratories and very few studies involving both rotavirus and enterotoxigenic bacteria in acute sporadic gastroenteritis of infants have been reported (Davidson *et al.* 1975; Echeverria, Blacklow & Smith, 1975; Ryder *et al.* 1976; Kapikian *et al.* 1976). Only one of these studies (Ryder *et al.* 1976) was carried out in a developing country, namely Bangladesh.

In South African Black population, and probably in many other developing communities throughout the world, childhood diarrhoea is of the greatest importance during the summer months (Spencer & Coster, 1969). In this report we present the bacteriological and virological findings in acute summer gastroenteritis in Black children under the age of 2 years admitted to hospital with severe dehydration.

MATERIAL AND METHODS

Patients

The patient sample consisted of 37 Black children (19 male and 18 female) admitted to Kalofong Hospital,Pretoria, between October 1975 and February 1976 suffering from severe gastroenteritis which necessitated intravenous rehydration. Their ages ranged from 17 days to 24 months (mean of 8·1 months) and they gave a history of diarrhoea and vomiting ranging from between 1 and 8 days (mean of 3·5 days). A history of previous diarrhoeal disease was obtained in 14 cases (38 %). In many cases it was difficult to determine whether the patients had been treated with antibiotics before admission. Witch-doctor preparations had been administered to 5 patients and another 5 had received antidiarrhoeal medication of an undetermined nature from their general practitioners.

Nutritional histories taken from the mothers revealed that only one child was being solely breast-fed. Of the remainder, 11 had never been breast-fed, 17 had been weaned at an average of 14 weeks, and 8 were being breast-fed with supplementary feeding.

Evidence of malnutrition was noted in 56 % of the patients, with weights below 80 % of the expected weight for age on the standard percentile charts. Diarrhoea symptomatic of other illnesses was excluded by clinical examination.

Collection of specimens

Faecal specimens were collected on the first day of admission by passing a No. 8 nasogastric feeding tube per rectum and aspirating rectal contents into a sterile 20 ml syringe. In most cases a copious amount of green watery stool could be easily obtained. Specimens were not sent on transport medium but reached the laboratory within 2 h of collection.

Bacteriological investigation

All faecal specimens were inoculated onto MacConkey, xyloselysine-deoxycholate, sodium chloride, and blood agar plates, and also into selenite broth. The agar plates were incubated for 48 h at 37 °C, except for one of the two MacConkey plates used per patient which was incubated for 72 h at room temperature (for *Yersinia enterocolitica*). The selenite broths were subcultured after 24 h incubation onto *Salmonella–Shigella* and brilliant green agar plates. From each patient between 10 and 23 colonies, both lactose and non-lactose fermenters, were subcultured and identified by the method of Edwards & Ewing (1972). A total of 720 colonies were thus examined. All isolates of *Escherichia coli*, *Salmonella* spp. and *Shigella* spp. were serotyped by slide agglutination using commercial antisera (Wellcome Reagents, Beckenham).

Identification of enterotoxigenic bacteria

All the bacterial strains (720 colonies) were examined for the production of heat-labile toxin (LT) and heat-stable toxin (ST). For screening purposes pools of five colonies were tested and if positive the individual members of each pool were tested separately. Ryder *et al.* (1976) have found that pools of ten colonies are satisfactory for screening purposes, as no individual strains were positive when the pools were negative.

The Chinese-hamster-ovary cell culture technique (Guerrant *et al.* 1974) was used to detect LT production. Cultures were inoculated into brain heart infusion broth (Difco) and incubated without shaking for 48 h at 35 °C. Sterile filtrates of this broth culture were added to the tissue culture immediately after subculturing the cells into 96-well microculture plates (Cooke). Six wells were used for each strain. These plates were incubated for 24 h at 37 °C in an atmosphere of 5% CO_2 in air and then examined with an inverted microscope. The percentage of elongated cells (the length greater than 3 times the diameter) in a total of 400 cells was recorded and values exceeding 15% were regarded as positive. All tests were performed in duplicate.

For detection of ST the infant mouse assay of Dean, Ching, Williams & Harden, (1972) was modified by using a thin plastic cannula to introduce the toxin into the stomach (Schoub *et al.* 1976*a*) instead of direct injection through the abdominal wall. The cultures were grown in brain heart infusion broth at 37 °C for 24 h on a shaking platform rotating at 180 rev./min. About 0·1 ml of sterile filtrate was inoculated into each mouse 3–5 days of age, and three mice were used per test. The mice were killed after 4 h and the intestine from pylorus to rectum was removed. The

gut:carcass weight ratio was determined and values exceeding 0·083 regarded as positive.

Identification of invasive bacteria

The technique of Serény (1955) was used. Cultures grown on nutrient agar slopes were washed off with phosphate-buffered saline, pH 7·2, to give a suspension of about 10^8 bacteria/ml. These suspensions were inoculated into the conjunctival sacs of adult guinea-pigs and then observed daily for 5 days for the development of kerotoconjunctivitis.

Virological investigations

Stool suspensions were examined for the presence of virus particles by negative staining electron microscopy (EM) as described previously (Schoub *et al.* 1975). Aqueous stool suspensions were clarified by centrifuging at 2500 rev./min for 30 min and the supernatant then concentrated by centrifuging at 100000 ***g*** for 1 h. The pellet was resuspended in distilled water and a drop of suspension was mixed with a drop of 3 % phosphotungstic acid at pH 6·0. After application to a formvar–carbon-coated copper grid the excess was removed with filter paper, allowed to dry, and the grid was then examined in a Philips EM 300 electron microscope at 60 kV.

The presence of rotavirus antigen was also investigated by a reverse complement fixation test (RCF) (Spence *et al.* 1975). A preparation of the patients' faeces was made by differential ultracentrifugation of an aqueous suspension of 1–5 g of stool, and this was then titrated against a fixed dilution of rabbit antiserum to the simian rotavirus SA11. (SA11 virus was kindly supplied by Dr H. H. Malherbe, Southwest Foundation, San Antonio, Texas). SA11 virus was used as a substitute antigen for the human agent for serodiagnosis because it is easily propagated and it is antigenically more closely related to the human virus (Schoub, Lecatsas & Prozesky, 1977) than the Nebraska calf diarrhoea virus used elsewhere (Kapikian *et al.* 1975). RCF titres exceeding 1/16 were regarded as positive.

RESULTS

Total laboratory findings

Enteric pathogens, bacterial or viral, were found in 30 out of 37 patients (81 %). In 7 of these 30 patients (23 %) a combination of bacterial and viral pathogens was found, and 2 patients had 2 different bacterial pathogens present in their stools. One patient was positive for rotavirus (by RCF) as well as for 2 different enterotoxigenic bacteria.

Bacteriological findings

Four strains of classical enteropathogenic bacteria were found in each of 4 patients: *Shigella flexneri*, *Shigella sonnei*, *Salmonella typhi* and *Salmonella paratyphi C*. In the 2 shigella patients this was the only pathogen isolated; however, the *S. typhi* patient was also rotavirus positive (by RCF) and the *S. paratyphi* patient also excreted LT-positive *Klebsiella pneumoniae*.

Table 1. *Enterotoxigenic bacteria isolated from patients with acute infantile gastroenteritis*

	LT	ST	Number of strains isolated
Escherichia coli	+	+	2
	+	−	2
	−	+	5
Klebsiella pneumoniae	+	−	4
Enterobacter cloacae	+	−	2
Proteus vulgaris	+	−	1

LT = Labile toxin.
ST = Stable toxin.

Table 2. *Association of enterotoxigenic bacteria with other enteric pathogens*

	Number of strains associated with:			
	Rotavirus	Other enterotoxigenic bacteria	*Salmonella* or *Shigella*	No. other enteric pathogens
E. coli LT+	0	0	0	3
E. coli ST+	1	1 (*K. pneumoniae*)	0	3
E. coli LT+ ST+	0	0	0	2
K. pneumoniae	2	1 (*E. coli* ST+)	1 (*S. paratyphi*)	0
E. cloacae	1	0	0	1
P. vulgaris	1	0	0	0

Enterotoxigenic bacteria were found in 15 of 37 patients (41 %) and one patient was positive for two different enterotoxigenic bacteria (*E. coli* ST+ and *K. pneumoniae* LT+) as well as rotavirus positive by RCF. The distribution of the enterotoxigenic bacteria is shown in Table 1 and their association with other enteric pathogens in Table 2. It is interesting to note that of 9 enterotoxigenic *E. coli* patients only 2 had concomitant infections with other enteric pathogens, whereas of the 7 strains of other enterotoxigenic bacteria 6 were associated with other pathogenic agents.

Invasiveness was demonstrated in the 2 shigella isolates but not with any of the other bacteria. One patient who was negative for bacteria and viruses yielded a heavy growth of *Candida albicans*, an organism which has been casually associated with enteritis (Kane, Chretien & Garagusi, 1976).

Virological findings

Rotavirus particles were detected by negative staining EM in only 2 patients (6 %), one of whom was also positive for *K. pneumoniae* LT+. With the RCF test 2 patients' specimens were anti-complementary; of the remaining 35 patients, 17 were positive (49 %) including the 2 specimens which were positive by electron microscopy.

When the patients were divided into 2 groups on the basis of the history of the

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duration of illness before admission, we found paradoxically that the longer duration groups (4 days and over) had twice as many RCF positive results as the shorter duration group (1–3 days), namely 66% and 33% positive respectively.

Bacterial pathogens were associated with 7 of the 17 rotavirus patients: *S. typhi* (1); *E. coli* ST+ (1); *E. coli* ST+ (1); *E. coli* ST+ and *K. pneumoniae* LT+ (1); *K. pneumoniae* LT+; *Enterobacter cloacae* (1); *Proteus vulgaris* (1). (One patient was positive for *E. coli* ST+ and *K. pneumoniae* LT+, as well as for rotavirus.)

Enteropathogenic serotypes of E. coli

Classical enteropathogenic serotypes of *E. coli* were found in 17 patients but only 6 of these were not associated with another pathogen. Serotype O125 K70 was found in 7 patients and O126 K71 in 3 patients. Enterotoxigenicity was detected in only 3 of the 18 seropositive strains. O55 K59 was LT+ ST+; O78 K89 was LT+; and O125 K70 was ST+.

DISCUSSION

Enteric pathogens were demonstrated in 81% of our patients with summer diarrhoea by combining classical diagnostic laboratory techniques with procedures to detect enterotoxigenic and invasive bacteria and viral pathogens in faecal specimens.

The rate of isolation of enterotoxigenic bacteria, 41%, appears to be similar to that found in developing communities elsewhere in the world: for example, 55% in Bangladesh (Nalin *et al.* 1975) and 50% in Brazil (Guerrant, Moore, Kirschenfeld & Sande, 1975). This contrasts with the failure to isolate enterotoxigenic bacteria from cases of infantile gastroenteritis in the developed countries such as Britain (Gross, Scotland & Rowe, 1976) and Australia (Davidson *et al.* 1975). From the United States divergent results have been published, varying from 73% in Chicago (Gorbach & Khurana, 1972) and 86% in Texas (Rudoy & Nelson, 1975) to 16% in Apache children (Sack *et al.* 1975) and zero in Honolulu (Dean *et al.* 1972), Boston (Echeverria *et al.* 1975) and the District of Columbia (Kapikian *et al.* 1976). The reason for these apparently conflicting figures in the United States is not clear. The isolation of LT-only and ST-only secreting strains of enterotoxigenic *E. coli* suggests that both enterotoxins are of importance in the aetiology of enteritis.

In common with Wadström *et al.* (1976) we have isolated enterotoxigenic bacteria other than *E. coli*, although in most cases these were associated with other enteric pathogens. It is thus insufficient to test only *E. coli* strains for enterotoxin secretion when investigating cases of enteritis. This has prompted us to reverse the conventional sequence of laboratory procedures used to diagnose enterotoxigenic bacteria. Rather than the initial biochemical tests for species identification of of isolated bacteria being followed by testing for enterotoxigenicity, we have now decided to test 15–20 random colonies first for enterotoxigenicity, followed by species identification of any colonies found to be positive.

Invasive *E. coli* have been reported from a few surveys of infantile gastroenteritis for example, 30% in Texas (Rudoy & Nelson, 1975), 10% in Brazil (Guerrant

et al. 1975) and 1% in Johannesburg, South Africa (R. M. Robins-Browne, personal communication). In common with the majority of reports on infantile gastroenteritis, however, we were unable to demonstrate the presence of invasive *E. coli*.

The value of serotyping *E. coli* in cases of sporadic infantile gastroenteritis remains a vexing question with workers in the field denying (Sack, 1976) as well as supporting (Rowe, Gross & Scotland, 1976) its usefulness. In common with the former group we found no correlation between the serotypes tested and enteropathogenicity, although in a previous survey (Schoub *et al.* 1975) we showed a statistically significant difference between patients and matched controls in the isolation of seropositive *E. coli*.

In general, rotavirus is the most important single aetiological agent of infantile gastroenteritis, having been detected at rates varying from 16% in Argentina (Lombardi, Roseto, Stamboulian & Oro, 1975) to 90% in Japan (Konno, Suzuki & Ishida, 1975). The most commonly reported prevalence rate is approximately 50%. Few studies have been carried out in developing countries. In Rhodesian Black infants rotaviruses were demonstrated in 44% of patients (Cruickshank, Zilberg & Axton, 1975) and in a previous study we found rotavirus by EM in 19% of Black patients in Johannesburg (Schoub *et al.* 1975). Our very low figure of 6% EM-detectable rotavirus infections in the present survey was surprising. At first we thought that this could be explained by seasonal variation, as workers elsewhere have reported a lower incidence in the summer months (Davidson *et al.* 1975; Kapikian *et al.* 1976). However, a more important reason became apparent when we examined the relation of timing of specimen collection to the rate of rotavirus detection by EM (Schoub *et al.* 1976*b*). It has been shown that the degree of viral shedding is maximal 2–3 days after onset of symptoms, after which it rapidly falls below the level of EM detectability of about a million particles per ml (Davidson *et al.* 1975). With the RCF test many more patients were rotavirus positive. The fact that the group of patients with a longer history of duration of symptoms had twice as many positives than the shorter history group leads us to suspect that the history of the duration of the illness was unreliable. The date of onset of the illness was possibly mistakenly taken as the date of onset of severe symptoms with the preceding milder symptoms being ignored.

Therefore in assessing the relative importance of bacterial and viral pathogens as aetiological agents in developing countries, the following should be taken into account:

(1) A falsely low rate of rotavirus detection by EM may be the result of patients reporting late for treatment.

(2) The relatively greater importance of enterotoxigenic bacteria when compared with developed countries.

Although workers in Bangladesh (Ryder *et al.* 1976) have obtained a correlation between clinical presentation of the patient and the presence of bacterial or viral enteric pathogens, we found no appreciable clinical difference.

It has long been known that breast-fed babies are far less subject to gastroenteritis than are those which are bottle-fed. It was interesting to note that only one of our patients was solely breast-fed and no pathogen was found. However, amongst

random age-matched children attending a surgical outpatients clinic 16% were solely breast-fed. A further eight gastroenteritis patients were breast- and bottle-fed and the isolation rate of viral and bacterial pathogens was not appreciably different from those patients solely bottle-fed. This may possibly indicate that the very much lower rate of gastroenteritis amongst breast-fed infants may be due to decreased opportunity for infection rather than a specific protective function of milk.

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