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Bacterial, parasitic and viral enteropathogens associated with diarrhoea in Saudi children

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SUMMARY In a 2-year study of stools from Saudi children with gastro-enteritis and from controls, rotavirus was the pathogen most frequently detected, either alone (44·3%) or in combination with other enteropathogens (7%). There were two peaks for rotavirus isolates, one during the cold months and the other during the dry, hot season. *Salmonella* spp. and *Campylobacter jejuni* were the second and third most frequently isolated enteropathogens. Enteropathogenic *Escherichia coli* did not contribute significantly to diarrhoea. Detection of enterotoxigenic *E. coli* was not attempted and its role in diarrhoea remains obscure. *Giardia lamblia* was detected more frequently in controls than in cases of diarrhoea. Neither *Entamoeba histolytica* nor *Schistosoma mansoni* was detected in any of the children studied.

Introduction

It is now well established that diarrhoea, mainly in children, is the major cause of morbidity and mortality in developing countries (1,2). In addition to rotaviruses, which are now recognized as the most common cause of diarrhoea among infants and young children in both developed (3–5) and developing countries (6,7), new enteric pathogens—*Campylobacter jejuni* (8), enteropathogenic *Escherichia coli* (EPEC), enterotoxigenic *Escherichia coli* (ETEC) and *Yersinia enterocolitica* (9,10)—have recently been recognized. Virtually no information is available on the incidence of diarrhoea caused by these enteric pathogens in Saudi children. In order to obtain this information, stool specimens from Saudi children with gastro-enteritis and from controls were investigated for bacterial, parasitic and viral agents during the course of a 2-year period (January 1985–December 1986).

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Materials and methods

Study area, patients and specimens

The patients and the controls studied were from Riyadh, the capital of Saudi Arabia, a subtropical area with an annual mean temperature of 26·5°C and a relative humidity of 24%. The area has a dry season from July to September and a cold season from January to April. The investigation involved Saudi children with gastro-enteritis under the age of 2 years admitted to King Khalid University Hospital and Sulaimaniah Children's Hospital in Riyadh between January 1985 and December 1986. The matched controls were children under 2 years of age admitted for illness other than gastro-enteritis. On the day of admission and on

three subsequent occasions, faecal specimens were collected and within hours were examined for bacterial and parasitic agents. A portion of each specimen was kept at −20°C to be examined later for viral agents.

Laboratory investigations

1. Bacteriology. Specimens were plated on blood agar, MacConky's agar, deoxycholate citrate agar (DCA), cefsulodin-irgasan-novobiocin (CIN) agar, Skirrows selective medium and in selenite broth for enrichment. After 24 h of incubation at 37°C the selenite broth was plated on MacConky's and xylose-lactose sodium deoxycholate agar (XLD). Suspicious colonies of non-lactose-fermenting organisms were first screened on urea slopes and triple sugar iron (TSI) for the possibility of shigella and salmonella. These were later confirmed by biochemical profile, using API-20 test (Analytical Profile Index—U.S.A.) and serological reactions.

For isolation of enteropathogenic strains of *E. coli* (EPEC), five to six random colonies of *E. coli* were picked up from MacConky's plate, screened by slide serologic reactions for enteropathogenic strains and confirmed by the tube titration test.

The Skirrows selective media for *Campylobacter jejuni* were incubated for 48 h under microaerophilic conditions using an oxoid campylobacter gas pack. As a control for microaerophilic conditions, a blood agar plate with *Pseudomonas aeruginosa, Clostriidium perfringens* and laboratory strain of *Campylobacter jejuni* was always used. Positive cultures were identified on the basis of their typical curved shape morphology, oxidase-positive test, hippurate hydrolysis, sensitivity to naladixic acid (30 μg) and resistance to cephalothin (30 μg).

The CIN plate was incubated for 24 h at 37°C and carefully checked for growth of typical "red bull's eye" colonies of *Yersinia enterocolitica.*

2. Parasitology. Fresh stools treated with saline and iodine preparations were examined for intestinal parasites. Samples containing trophozoite and/or cyst stages of *Entamoeba histolytica, Giardia lamblia* or any other parasites were considered positive.

3. Virology. Stool specimens were screened for the presence of rotavirus by the enzyme-linked immunosorbent assay (ELISA) technique using the commercially available kit from Abbott Laboratories, North Chicago, Illinois (Rotazyme Diagnostic kit). Stool specimens which were negative for rotavirus by ELISA were investigated for other viruses by the electron microscope.

The rates of infections were compared using the Chi-squared test, except when numbers were low, when Fisher's Exact Probability test was used.

Results

The enteropathogens detected in the stools of children with gastro-enteritis and in controls are shown in Table I. Rotavirus was the most frequently identified enteropathogen in gastro-enteritis cases (44·3%) and in controls (28·1%), followed by *Salmonella* spp (8·0% cases; 2·9% controls), enteropathogenic *E. coli* (3·9% cases; 3·5% controls), *Campylobacter jejuni* (2·9% cases; 1·0% controls) and *Shigella* spp (1·0% cases; none detected in controls). The association of gastro-enteritis with rotavirus ($P < 0{\cdot}001$) and salmonella ($P < 0{\cdot}001$) was statistically significant in contrast with the association with enteropathogenic *E. coli* ($P > 0{\cdot}2$). *Giardia lamblia* was isolated more frequently in the controls than in gastro-enteritis ($P < 0{\cdot}05$).

Table II shows the number of specimens which contained more than one enteropathogen. Rotavirus was the most frequent enteropathogen detected as a single agent (Table I) or in association with other enteropathogens.

The seasonal pattern of the two most commonly detected enteropathogens (rotavirus and salmonella) is shown in Figure 1. Although rotavirus could be detected all year round, two peaks for the highest incidence of rotavirus isolation were noted: one during the colder months of the year (February–March) and one during the dry months of the year (July–September). No definite seasonal pattern of infection by the other enteropathogens could be seen.

Discussion

This study, which covered a 2-year period, is the first inventory of the different enteropathogens associated with diarrhoea in infants and children in Saudi Arabia. Rotavirus was the pathogen most frequently detected either as a single agent (44·3%) or in combination with other enteropathogens (7%). This is in agreement with other studies from many developed (5,11) and developing countries (12–20). The incidence of rotavirus reported in several developing countries varies from 15% in the Philippines (12) to 17·6% in the Central African Republic (13), 27·8% in Addis Ababa, Ethiopia (14), 40% in Zimbabwe (15), 50% in

Table 1 Occurrence of bacteria, viruses and parasites in faeces of 688 children with gastro-enteritis and in 512 controls

Enteropathogens	Gastro-enteritis No.	%	Control No.	%	Significance
Bacteria					
Enteropathogenic *E. coli*	27	3·9	18	3·5	$P > 0{\cdot}2 \times 0{\cdot}83$(xc)
Salmonella	55	8	15	2·9	$P < 0{\cdot}001$
Salmonella spp.	(54)		(15)		
Salmonella paratyphi B	(1)		(0)		
Shigella	7	1	0	0	$P = 0{\cdot}04^{*} \times 0{\cdot}04$
Shigella flexneri	(5)		(0)		
Shigella sonnei	(2)		(0)		
Campylobacter jejuni	20	2·9	5	1	$P < 0{\cdot}05$
Yersinia enterocolitica	0	0	0	0	
Viruses					
Rotavirus	305	44·3	144	28·1	$P < 0{\cdot}001 \times 10^{-6}$
Other viruses**	2**	0·3	1***		
Parasites					
Giardia lamblia	11	1·6	18	3·5	$P < 0{\cdot}05$
Total	427	(62·1)	201	(39·3)	

* Fisher's Exact Probability test;
** 1 calicivirus and 1 astrovirus;
*** adenovirus

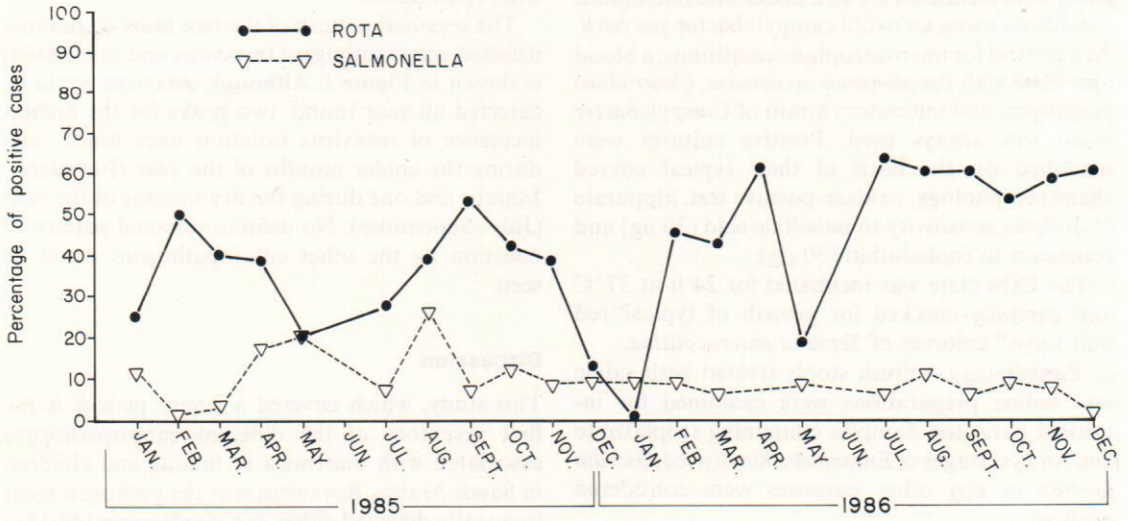

Figure 1 Seasonal fluctuations in the incidence of Rotavirus and Salmonella in Riyadh, Saudi Arabia from January 1985 through December 1987.

Kenya (16), 50% in Bangladesh (17), 61% in Nigeria (18) and 70% in a South Indian town (19). The incidence of rotavirus reported from neighbouring Kuwait was 24·5% (20).

In temperate climates, the highest incidence of rotavirus infection is during the winter months of the year (21). This pattern, however, varies in tropical countries and in areas with fluctuating weather conditions (14,22,23). It seems that there are two peaks of rotavirus infection in

Table II More than one enteropathogen detected in the same stool specimen

Enteropathogen	Gastro-enteritis No.	%	Control No.	%
Rotavirus + EPEC	14	2	3	0·6
Rotavirus + salmonella	20	2·9	5	1
Rotavirus + shigella	3	0·4	0	0
Rotavirus + *Campylobacter jejuni*	6	0·9	4	0·8
Rotavirus + *G. lamblia*	4	0·6	5	1
Rotavirus + *G. lamblia* + *Campylobacter jejuni*	1	0·15	0	0
G. lamblia + EPEC	1	0·15	0	0

Riyadh, Saudi Arabia: one during the cold months (January–April) and the other during the dry months of the year (July–September). A similar pattern was noticed in Addis Ababa (14). In South American countries, rotavirus infection was readily detected throughout the year in Bele'm, Brazil (24) and in Guatemala (25), but tended to occur during the cooler months of the year in Venezuela (26).

After rotavirus, salmonella was the most frequent enteropathogen isolated in our patients (8% cases; 2·9% controls) and the association of salmonella with diarrhoea was statistically significant ($P < 0{\cdot}001$). Mixed infection by salmonella and rotavirus was also relatively frequent in our study (2·9%). It remains to be determined which enteropathogen of these two was primarily implicated in the aetiology of diarrhoea and if both enteropathogens act synergistically to produce the disease. The incidence of shigellosis was low in our study (1·0%), in contrast with some neighbouring countries such as Egypt where it appears to be the leading cause of gastro-enteritis (27).

Campylobacter jejuni was the third most frequently isolated enteropathogen (2·9% cases; 1·0% controls). Although this pathogen seems to play a role as an aetiological agent of diarrhoea in our children ($P < 0{\cdot}05$), it does not seem to have a seasonal pattern in contrast with the situation in other tropical countries (13,28). No *Yersinia enterocolitica* was isolated in this study. This could be due to the tropical climatic condition of our area.

Although there is evidence that enteropathogenic *E. coli* is an important pathogen in many developing countries (13,17,25,27), the association of EPEC with diarrhoea in our children was not statistically significant ($P > 0{\cdot}2$) and this was irrespective of the type of strains isolated. In San Paulo, Brazil (29), EPEC strains were the most frequent cause of diarrhoea in children 0–5 months old. Enterotoxigenic *E. coli* also has been reported to be the most frequent pathogen identified in many developing countries (17,30). Detection of this enteropathogen, however, was not attempted in this study and its role as a causative agent of diarrhoea in Saudi children is still to be determined.

Giardia lamblia in this study was isolated in controls more often than in the cases. This, however, is not unusual in many tropical and subtropical countries where the majority of infected children are asymptomatic (31). Furthermore, neither *Entamoeba histolytica* nor *Schistosoma mansoni* was detected in our children. This could be due to the young age of the population studied (2 years) as these two parasitic enteropathogens were found to be frequently associated with diarrhoea in children over 2 years of age (13).

The high frequency of the various enteropathogens and the phenomenon of mixed infection observed suggest that diarrhoeal disease in Saudi Arabia is a complex and multifaceted problem. Further investigations are needed and these include, among others, the prevalence of the various serotypes of rotavirus, the pathophysiology of mixed infections and, more important, the clinical trials of different therapies for dehydration.

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