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Detection of coronaviruses in children with acute gastro-enteritis in Maddina, Saudi Arabia

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Abstract

Background: The role of coronaviruses in paediatric gastro-enteritis is not well defined. We investigated the detection rate and epidemiological features of infection with coronavirus in children receiving hospital care for acute gastro-enteritis in Maddina, Saudi Arabia.

Methods: Stool specimens were collected from children less than 5 years of age who were either hospitalised in Maddina or given oral rehydration therapy as outpatients between April 2004 and April 2005. Coronaviruses were detected by electron microscopy.

Results: Coronaviruses were detected in 63 (6%) of 984 children with acute gastro-enteritis and were more commonly detected in outpatients (47/423, 11%) than in inpatients (16/561, 3%). The median age (range) of children with coronavirus infection was 42 months (10–60). Coronaviruses were detected throughout the year with the highest detection rate at the end of the winter season.

Conclusions: Coronaviruses were commonly identified in children with diarrhoea in Saudi Arabia. Their role in paediatric gastro-enteritis warrants further evaluation.

Introduction

Diarrhoeal disease remains a major cause of childhood mortality, annually accounting for more than 1.8 million deaths globally in children under 5 years of age.[1] Worldwide, rotavirus is the most important cause of severe, dehydrating gastro-enteritis in infants and young children.[2] Other established viral causes of paediatric gastro-enteritis include astrovirus, enteric adenovirus, norovirus, and sapovirus.[3]

Coronaviruses are single-stranded RNA viruses that belong to the Coronaviridae family.[4] The virus particle is 60–220 nm in size and has a helicoidal symmetry with a spiculated envelope that gives the appearance of a crown. Coronaviruses that cause respiratory illness in humans (principally the common cold) are termed respiratory coronaviruses. The genetically distinct gastro-intestinal coronaviruses have long been recognised as important causes of gastro-enteritis in animals, although the role of coronaviruses as aetiological agents of gastro-enteritis in humans is much less well established.[4,5] The emergence of the severe acute respiratory syndrome (SARS) was associated with a novel coronavirus, SARS-CoV,[4,5] and SARS-CoV was noted to be

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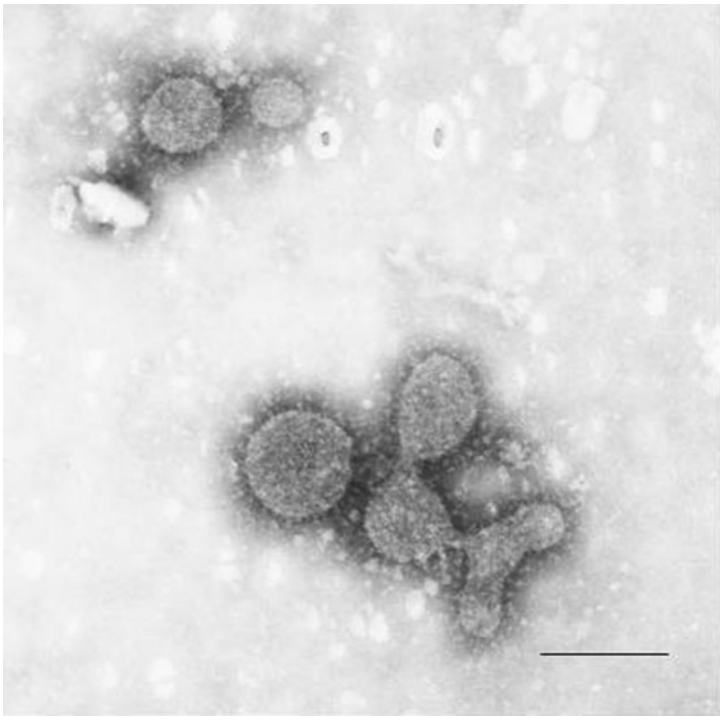

FIG. 1. Negatively-stained electron micrograph of coronavirus particles in a faecal specimen. Potassium phosphotungstate stain. Bar represents 100 nm in length.

associated with significant gastro-intestinal disease.[6]

In a recently completed 1-year study of rotavirus gastro-enteritis in children in Maddina, Saudi Arabia, rotavirus was not identified in 81% of cases.[7] We therefore subjected all faecal samples to electron microscopy (EM) examination in order to detect any other viral enteropathogens. Unexpectedly, coronavirus was observed relatively frequently.

Subjects and Methods

Subject enrolment and specimen collection was undertaken at the Maternity and Children's Hospital and Ohod Hospital, Maddina, Saudi Arabia, as described previously.[7] Briefly, stool specimens were collected from children under 5 years with acute gastro-enteritis who had been referred to the oral rehydration unit (outpatients, $n=423$) and admitted to the hospital (inpatients, $n=561$) during a 1-year period between 17 April 2004 and 16 April 2005. All specimens were stored at -20°C until being shipped to the Division of Medical Microbiology, University of Liverpool.

All faecal samples were subjected to EM examination. The procedure has been described previously[8] and was performed using a Philips 301 electron microscope (Philips Electron Optics UK Division, PYE Unicam Ltd, Cambridge, UK) at screen magnification of $\times 45{,}000$. Coronaviruses were identified by their characteristic size and morphology (Fig. 1).

Statistical tests were performed using EpiInfo version 6 (CDC, Atlanta, GA, USA). The detection rate of coronavirus was compared between groups using the χ^2 test. A p-value of <0.05 was considered significant.

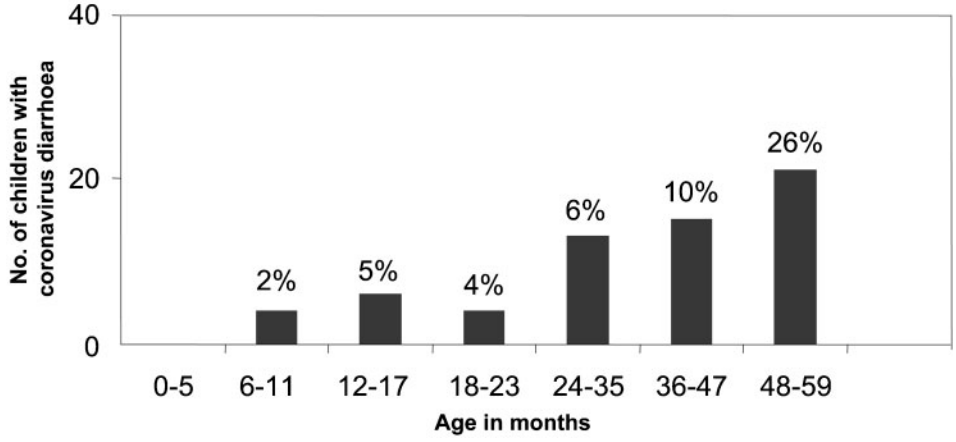

FIG. 2. Age distribution of coronavirus infection in children with diarrhoea. The percentage shown above each bar indicates the detection rate of coronavirus in all diarrhoeal cases in the indicated age group.

Results

Coronaviruses were identified in 63/984 (6%) stool specimens and were significantly more common in outpatients (47/423, 11%) than in inpatients (16/561, 3%) ($p<0.01$). The median age (range) of children with coronavirus infection was 42 months (10–60) compared with 21 months (0.5–60) in uninfected children. Among the coronaviruses identified, 78% (49/63) were in children over 2 years of age (Fig. 2). Coronaviruses were detected in each month of the year, the monthly detection rate ranging from 2% to 13%. Forty-nine per cent (31/63) of coronaviruses were detected in a 3-month period between January and March, coinciding with the end of winter in Maddina (Fig. 3).

Discussion

While the role of rotavirus as a causal agent of gastro-enteritis has been previously described in Saudi Arabia,[7,9–11] little is

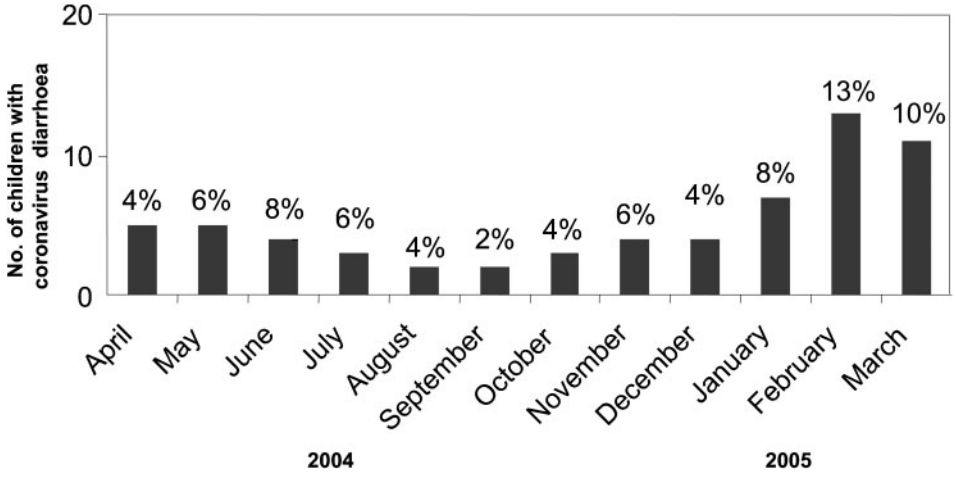

FIG. 3. Monthly detection of coronavirus in children <5 years of age with diarrhoea. The percentage shown above each bar indicates the detection rate of coronavirus in all diarrhoeal cases in the month indicated.

known about the importance of other gastro-intestinal viruses. Using EM, we detected coronaviruses in 6% of children with diarrhoea. A single previous study from Saudi Arabia reported a very low detection rate of 0.3%.[12]

Several epidemiological features of coronavirus infection in this population contrast sharply with that of rotavirus infection which we described previously.[7] Firstly, most coronaviruses were identified among outpatients, suggesting that coronavirus infection may more often result in less severe diarrhoea not requiring hospitalisation. This observation is consistent with previous studies which have generally documented that coronavirus diarrhoea is usually mild (Table 1).[12–25] Secondly, the median age of children with coronavirus infection (42 months) was significantly greater than in children with rotavirus infection (10 months),[7] which raises the possibility of age-dependent differences in susceptibility to infection, or perhaps different modes of virus transmission. Thirdly, the seasonality of coronavirus infection differed from that of rotavirus; rotavirus infection was most common between November and January whereas the highest monthly detection rates of coronavirus were between January and March. Although firm conclusions regarding seasonality cannot be drawn from a single year of study, differences in the detection rate of coronavirus by age and season when compared with rotavirus have been noted previously.[17]

The detection rate of coronavirus in this study (6%) is greater than in many but not all previous studies of paediatric gastro-enteritis in which detection rates ranged from zero to 38% (Table 1). It is noteworthy that a study from a neighbouring country, Iran, reported that coronavirus ranked second (3%) to rotavirus as a cause of childhood viral gastro-enteritis.[25] However, while in some studies coronavirus has been associated with diarrhoea,[13,18,19,25] other studies have found coronavirus equally common in patients without diarrhoea.[15–17] Thus, the aetiological role of coronaviruses in human gastro-enteritis is not firmly established.

TABLE 1. *Summary of studies which have investigated the role of coronavirus in paediatric gastro-enteritis.* *

		Study characteristics				Coronavirus detection (%)		
Country	Year	Duration, mths	Setting	No. of subjects	Age, yrs	Cases (diarrhoea)	Controls (no diarrhoea)	Ref.
UK	1975–76	23	IP, OP	230	<1	0	0	13
				227	1–14	2	0	
USA	1976–84	96	IP	862	ANI	28	ND	14
Vanuatu	1979–80	13	IP	22	<5	23	23	15
Gabon	1980–81	12	OP	56	<10	38	65	16
India	1981–82	24	IP	426	<12	9	23	17
Italy	1981–83	24	IP, OP	208	<4	16	2	18
Italy	1982–84	24	IP	561	6–24 mths	2	ND	19
Mozambique	1985–86	12	IP	310	<2	2	2	20
UK	1985–87	36	IP, OP	NR	ANI	1	NR	21
Brazil	1988–89	13	IP	67	<2	0	0	22
Saudi Arabia	1990–93	36	IP, OP	7439	I & C	0.3	ND	12
UK	1999–01	24	IP, OP	271	<5	0	ND	23
Australia	2000	12	IP, OP	412	<6	0	0	24
Iran	2001–02	11	IP, OP	504	<5	3	0	25
Range	1975–02	11–96		22–7439		0–38	0–65	

* All these studies used EM to detect viral enteropathogens. NR, not reported; ND, not done; IP, hospital inpatients; OP, hospital outpatients; ANI, age not identified; I, infants; C, children.

Recent studies of coronavirus diarrhoea in humans (excluding that associated with SARS-CoV) are few and most date back to the 1970s and '80s. Most likely, this is because the diagnosis of gastro-intestinal coronavirus infection still relies upon EM. This technique requires expensive equipment and maintenance, and a highly skilled operator. Thus, in contrast with other enteric viruses (e.g. rotavirus, norovirus, astrovirus) which have been subjected to extensive investigation using sensitive, modern serological and molecular tools, the gastro-intestinal coronaviruses have been relatively neglected. This is likely to have resulted in under-estimation of the burden of disease attributable to them. It is of note that the application of sensitive assays to detect multiple viruses, bacteria and parasites among children with diarrhoea still leaves a 'diagnostic gap' of around 31.4%.[26] Given the limitations of diagnosis by EM, the development and application of more sensitive molecular assays to detect the gastro-intestinal coronaviruses will lead to better understanding of their role in gastroenteritis in both children and adults, and may allow the diagnostic gap to be closed further.

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