Epidemiology and microbiology of diarrhoea in young Aboriginal children in the Kimberley region of Western Australia

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INTRODUCTION

Infectious diarrhoea is common in young Australian Aborigines [1-3] and is one of the main causes for their unsatisfactory health standards with consequent widespread failure to thrive and undernutrition [4-5]. Most published reports relate to patients in hospital or to hospital admission statistics and give little indication of the extent or severity of diarrhoeal disease in children in Aboriginal communities.

The present investigation involved more than 100 Aboriginal children up to 5 years of age living in remote communities in the tropical north of Western Australia who were studied prospectively over a 12-month period.

METHODS

Subjects and samples

Faecal specimens were obtained from 104 Aboriginal children up to 5 years of age in the course of community surveys in the early wet (Nov.—Jan.), late wet (Feb.—Apr.), early dry and late dry (Aug.—Oct.) seasons in this tropical location where the summer monsoon usually coincides with the highest annual incidence of diarrhoea. Samples were collected by a community health nurse and Aboriginal health workers over 3 consecutive days. Specimens were placed into sterile, screwtopped plastic containers and inoculated into Cary-Blair transport medium, and shipped overnight by air to Perth where they were cultured within 24 h.

Diarrhoea was defined as the passage of three or more abnormally loose stools per day. The decision as to whether diarrhoea was or was not present was made after discussions with the mother by the community health nurse and by observations of the stools.

The children were weighed on standardized Seca beam balance scales (to 10 g), and recumbent length (to 24 months) or height (2–5 years) measured to 5 mm with specially designed boards using standard techniques. [6–7]. Standard deviation (Z) scores relative to international reference values [8] were used to classify

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nutritional status; children whose Z scores decreased by more than 1·0 negative points between 6 and 24 months of age were deemed to have 'inadequate' growth while those whose change in Z scores were above this cut-off level or had stationary Z scores were classified as having 'satisfactory' growth.

$Microbiological\ methods$

Isolation and primary identification of bacterial pathogens were performed according to Edwards and Ewing [9]. Faecal specimens were streaked onto MacConkey agar plates which were incubated overnight at 37 °C. Five lactose-fermenting colonies, typical of $Escherichia\ coli$, were isolated from each plate for further testing. Strains were confirmed as $E.\ coli$ by biochemical reactions [10] and lactose-negative colonies were studied biochemically and kept for further testing if found to be $E.\ coli$.

 $E.\ coli$ strains were blotted onto Zeta Probe membranes, each of which was hybridized with DNA probes to detect strains which were enterotoxigenic (LT, ST-Ia and ST-Ib), verotoxigenic (VT-1 and VT-2), enterohaemorrhagic (EHEC) and enteropathogenic (EPEC). Strains resembling an EIEC [11] were immediately tested for invasiveness by the Sereny test. All $E.\ coli$ strains were stored in maintenance medium and any strains hybridizing with a gene probe were frozen at $-70\ ^{\circ}$ C in 1 ml volumes of glycerol broth [12].

Faecal samples were plated onto desoxycholate citrate and xylose lysine desoxycholate and incubated overnight for isolation of Salmonella species, Shigella species or $Edwardsiella\ tarda$. Faeces were also directly inoculated into Selenite F enrichment broth and incubated at 43 °C for 20–24 h. Subcultures from the broth were made on desoxycholate citrate and bismuth sulphite agar plates. Salmonella strains were further characterized by agglutination with specific antisera. Shigella strains were identified by biochemical and serological reactions; $E.\ tarda$ was identified by biochemical reactions [6]. Campylobacter species were cultured on Skirrow's medium which was incubated at 43 °C under reduced oxygen partial pressure; species were identified by their growth characteristics, appearance after staining, biochemical reactions and antimicrobial sensitivity [13]. Aeromonas species were isolated on blood agar/ampicillin plates [14]; colonies showing β -haemolysis were tested for oxidase activity [15].

Microscopy of faecal specimens was used to identify the presence of erythrocytes and leukocytes, Giardia lamblia trophozoites, and other parasites including Hymenolepis nana, S. stercoralis, A. duodenale, B. hominis and Entamoeba coli. Faecal emulsions were also examined after concentration by centrifugation. Samples were examined for Cryptosporidia by safranin/methylene blue staining [16].

Rotavirus was detected in faecal suspensions using a specific latex agglutination test kit (RotaScreen®).

RESULTS

Rates of diarrhoeal episodes

A total of 555 faecal samples was obtained from 104 Aboriginal children of 5 years of age and less who lived in remote communities in the Kimberley region of Western Australia. The number of samples obtained from children in each age

Table 1. Age distribution and diarrhoeal status of samples

	No. of samples (%)		
Age (months)	Diarrhoeal	Non-diarrhoeal	
0-5.9	26 (36·1)	46 (63.9)	
6-11.9	50 (37.3)	84 (62.7)	
12 - 17.9	43 (36.8)	74 (63.2)	
$18 - 23 \cdot 9$	28 (37.3)	47 (62.7)	
$24 - 35 \cdot 9$	17 (27.9)	44 (72.1)	
> 36	9 (9.4)	87 (90.6)	
Total	173 (31.2)	382 (69.8)	

Table 2. Seasonal variation and diarrhoeal status of samples

	No. of samples (%)		
Season (months)	Diarrhoeal	Non-diarrhoeal	
Early wet (NovJan.)	54 (47.8)	59 (52.5)	
Late wet (FebApr.)	33 (31.7)	71 (68.3)	
Early dry (May-July)	45 (23.4)	147 (76.6)	
Late dry (AugOct.)	41 (28.1)	105 (71.4)	

group is shown in Table 1. A total of 173 specimens were taken from children who were considered to have an acute episode of diarrhoea at the time of sampling.

The proportion of diarrhoeal specimens obtained from children in the 0–6 months age group was relatively high (> 36%) and this level was maintained until 2 years of age; thereafter a slight but not significant decline in the proportion of diarrhoeal samples ($P \ge 0.2$) was seen. However, there was a significant reduction in the proportion of diarrhoeal samples obtained from children over 3 years of age compared to younger children ($P \le 0.001$).

The proportions of diarrhoeal samples showed a significant seasonal variation (Table 2). Nearly half of the samples obtained during the early wet season (Nov.–Jan.) came from children with diarrhoea compared to the early dry season (May–July) when less than one-quarter of the children had diarrhoea at the time of sampling ($P \leq 0.02$). The proportion of diarrhoeal samples during the late wet (Feb.–Apr.), early dry and late wet (Aug.–Oct.) seasons (< 30%) did not differ significantly ($P \geq 0.1$). The increase in the proportion of samples from symptomatic children during the early wet season corresponded with the increased rainfall and highest average monthly maximal and minimal temperatures in the West Kimberley region during this season.

Faecal enteric pathogens

Enteric pathogens were identified in $65\cdot3$ % of faecal samples from children with diarrhoea and from $45\cdot3$ % of samples from asymptomatic children. There was no seasonal association with the isolation of enteric pathogens and diarrhoea. However, there was a significant association between diarrhoea and the presence of enteric pathogens in children up to the age of 2 years ($P \le 0.05$). Carriage of enteric pathogens was more frequent in asymptomatic children older than 2 years compared to younger asymptomatic children ($P \le 0.001$) (Table 3).

Table 3. Age distribution and diarrhoeal status of faecal samples shown to carry an enteric pathogen

	No. of samples (%)		
		~	
Age (months)	Diarrhoeal	Non-diarrhoeal	
0–6	18 (69.2)	15 (32.6)	
7–12	29 (58.0)	31 (36.9)	
13-18	29 (69.4)	26 (35.1)	
19 – 24	20(71.4)	23 (48.9)	
25 - 36	10 (58.8)	25 (56.8)	
> 36	7 (77.7)	53 (60.9)	
Total	113 (65.3)	173 (45.3)	

Table 4. Enteric pathogens

No.	of	iso.	lates	(%)	١

	Diarrhoea	No diarrhoea	P value
$E.\ coli$			
verotoxigenic	26 (15.0)	23 (6.0)	≤ 0.01
enterotoxigenic	28 (16.2)	34 (8.9)	≤ 0.05
enteroinvasive	0	0	
Salmonella spp.	22 (12.7)	28 (7.3)	≤ 0.05
Shigella spp.	8 (4.6)	6 (1.6)	≤ 0.01
$S.\ flex neri$	6(3.4)	5 (1.3)	
$S.\ sonnei$	2 (1.2)	1 (0.3)	
Campylobacter spp.	8 (4.6)	14 (3.7)	$\geqslant 0.2$
$C.\ jejuni$	8 (4.6)	13 (3.4)	
$C.\ coli$	0	1 (0.3)	
$A.\ hydrophila$	0	3(0.8)	
E. tarda	1 (0.6)	2 (0.5)	$\geqslant 0.2$
Rotavirus	12 (6.9)	3(0.8)	
Cryptosporidium	14 (8.1)	6 (1.6)	≤ 0.01
$G.\ lambli a$	12 (6.9)	34 (8.9)	$\geqslant 0.2$
H. nana	11 (6.4)	53 (13.9)	$\geqslant 0.2$
$S.\ stercoralis$	2 (1.2)	10 (2.6)	$\geqslant 0.2$
B. hominis	3(1.7)	8 (2.1)	$\geqslant 0.2$
$A.\ duodenale$	0	7 (1.8)	
$A.\ lumbricoides$	1 (0.6)	0	
T. trichuria	1 (0.6)	0	

The bacterial, viral and parasitic enteric pathogen isolations are shown in Table 4. Overall, isolation of bacterial or viral pathogens, was significantly associated with diarrhoea ($P \le 0.025$), while isolation of parasites and multiple enteric pathogens was not associated with diarrhoea ($P \ge 0.1$). The only viral pathogen looked for was rotavirus.

Isolations of bacterial, viral and multiple pathogens were associated significantly more often in children with diarrhoea than those who were well in the less than 2 years of age group ($P \le 0.025$) whereas in older children no such association was demonstrable ($P \ge 0.2$). There were no viral isolations in the older age group. Multiple infections in children less than 18 months of age were either multiple bacterial infections (51.4%) or bacterial-viral infections (20.0%). Such multiple infections were significantly associated with diarrhoea in this age group

Table 5. Salmonella serovars

No. of isolates (%)

Serovar	Diarrhoea (%)	No diarrhoea (%)	
S. abony	3 (1.7)	0	
S. anatum	1 (0.6)	2(0.5)	
S. arizona	0	1 (0.3)	
S. chester	3 (1.7)	2(0.5)	
S. eastbourne	1 (0.6)	0 `	
S. havana	3 (1.7)	1 (0.3)	
S. infantis	1 (0.6)	0 `	
S. kimberley	0 '	1 (0.3)	
S. litchfield	0	1 (0.3)	
S. oranienberg	0	1 (0.3)	
S. rubislaw	1 (0.6)	0 ` ′	
S. senftenberg	1 (0.6)	6 (1.6)	
S. urbana	1 (0.6)	0 ` '	
S. wandsworth	6(3.5)	13 (3.4)	
S. waycross	1 (0.6)	0 ` ′	

 $(P \le 0.001)$. The predominant forms of multiple infection in children older than 18 months were either bacterial-parasitic (59.0%) or parasitic-parasitic (26.2%) and were not associated with diarrhoea $(P \ge 0.9)$.

No age or seasonal association with parasite carriage was noted $(P \ge 0.125)$ although there was a slight increase in the proportion of diarrhoeal stools containing parasites during the wet season. In contrast, isolation of bacterial enteric pathogens was significantly associated throughout the year with diarrhoea and, furthermore, the proportion of bacterial pathogens detected in both asymptomatic and symptomatic faecal samples increased during the dry season. Isolations of multiple pathogens increased during the wet season but with no association with diarrhoea during any season. The numbers of viral isolations were too low to make a seasonal association.

Bacterial enteric pathogens

Of specific bacterial pathogens, only verotoxigenic and enterotoxigenic *E. coli*, *Salmonella* and *Shigella* spp. were significantly associated with diarrhoea (Table 4).

Fifteen different Salmonella serovars were identified (Table 5). S. wandsworth was the most common serovar identified, accounting for 38% of all Salmonella isolates, but was not significantly associated with diarrhoea. Similarly, the isolation rate of S. senftenberg from asymptomatic carriers was more than double that from symptomatic patients. The remaining Salmonella spp. were associated with diarrhoea ($P \leq 0.001$) although the association of individual serovars with diarrhoea could not be determined due to the small number of isolates.

There was no seasonal association between the presence of salmonella and diarrhoea but a significant increase in the isolation rate of *Salmonella* strains from both symptomatic and asymptomatic children occurred from February to July (11.5%) compared to the rest of the year (6.2%) ($P \le 0.03$). The presence of salmonellas in children less than 18 months of age was definitively associated with diarrhoea ($P \le 0.02$) but not in children over 18 months of age ($P \ge 0.08$).

	['] Diarrhoea	No diarrhoea	P value
$E.\ coli$			
verotoxigenic	13 (9.6)	11 (3.4)	≤ 0.01
enterotoxigenic	15 (11.0)	17 (5.2)	€ 0.03
Salmonella spp.	12 (8.8)	17 (5.2)	$\geqslant 0.2$
Shigella spp.	1 (0.7)	4 (1.2)	≤ 0.8
S. flexneri	0	3 (0.9)	
$S.\ sonnei$	1 (0.7)	1 (0.3)	
Campylobacter spp.	2(1.5)	8 (2.4)	$\geqslant 0.4$
C. j e j u n i	2(1.5)	7(2.1)	
$C.\ coli$	0	1 (0.3)	
$A.\ hydrophila$	0	1 (0.3)	
E. tarda	1 (0.7)	2 (0.6)	≥ 0.2
Rotavirus	5 (3.7)	2(0.6)	≤ 0.02
Cryptosporidium	9 (6.6)	3(0.9)	≤ 0.001
$G.\ lamblia$	6 (4.4)	8 (2.4)	≥ 0.1

4(2.9)

1(0.7)

0

Table 6. Enteric pathogens excluding multiple isolates
No. of isolates (%)

S. flexneri accounted for just over three-quarters of all shigella infections and S. sonnei the rest. In only one instance was the species alone associated with diarrhoea. Most (85·7%) shigella infections occurred in children less than 2 years of age. No child older than 2 years with diarrhoea had a Shigella species isolated from their faeces. The majority (78·6%) of shigella infections occurred during August to January and Shigella spp. isolated during the rest of the year came from asymptomatic children.

27 (8.3)

5 (1.5)

4 (1.2)

2(0.6)

≥ 0.8

≥ 0.2

Of the 21 Campylobacter sp. isolates, one was C. coli and the others C. jejuni. There was no age of seasonal association with campylobacter infection.

Parasites

H. nana

S. stercoralis

B. hominis

A. duodenale

Cryptosporidium was the only parasite to be significantly associated with diarrhoea. Most (85%) of the isolates occurred during the wet season, when the isolation rate of Cryptosporidium from symptomatic children outnumbered the isolation rate from asymptomatics by more than 3 to 1. Over 90% of Cryptosporidium isolates came from children less than 2 years of age. The highest incidence of infestation occurred in the 6–18 months old age group where 5·2% of faecal samples contained Cryptosporidium compared to 2·3% for the older age groups.

Neither H. nana (the most common parasite identified) nor G. lamblia was associated with diarrhoea. There was no significant seasonal association with infestation although the carriage rate for both parasites increased slightly during the dry season. Both were isolated more frequently in children over the age of 2 years ($P \leq 0.001$).

The remaining parasites including S. stercoralis, B. hominis, A. duodenale. A. lumbricoides and T. trichiura, were infrequently found and were generally not

associated with diarrhoea (Table 4). The small number of isolates makes it impossible to show any seasonal or age association with parasite infestation.

Multiple enteric pathogens

In view of the high rate of infection with multiple enteric pathogens, enteric pathogen isolations were re-examined and all multiple isolations were excluded in order to gain a general impression of the role of individual enteric pathogens as causes of diarrhoea (Table 6). Salmonella and Shigella species were the only enteric pathogens which had previously been associated with diarrhoea (Table 4) but when present alone were apparently not. However, the number of Shigella isolates was extremely small after the removal of multiple isolates. Over the 12-month period 12 children in the 'satisfactory' nutritional group and 22 in the 'inadequate' nutrition group had multiple faecal samples, taken at least at 1 month intervals, from which pathogens or parasites were isolated. Fifty-seven per cent of children with multiple positive samples had 2 faecal samples with pathogens or parasites isolated but the rest had up to 6 stool samples which were positive.

DISCUSSION

This study showed that diarrhoeal episodes were common in Kimberley Aboriginal children, particularly in the first 2 years of life, and were associated with the presence of intestinal parasites, and bacterial and viral pathogens in their stools. Over one-third of infants had episodes of diarrhoea in the first 6 months of life and rather more in the following three semesters; after 2 years of age the proportion fell significantly. These results compare with a prospective, community-based study of Kimberley Aborigines of whom 6% had diarrhoea recognized between birth and 6 months, 23% between 6 and 12 months and 46% during each of the two semesters of the second year of life (Gracey and colleagues, unpublished). Diarrhoeal illness was significantly more common during the early wet, summer season which is the hottest part of the year when monsoonal rains arrive from South East Asia. It is well recognized, locally, as the 'diarrhoea season' [17].

The seasonality of childhood diarrhoeal disease varies markedly from place to place. In temperate climates rotavirus infections tends to occur in peaks or epidemics in winter as in the United States where children between 6 and 36 months of age are predominantly involved [18]. Rowland and colleagues [19] found the association of childhood diarrhoea in The Gambia with the 5-month annual wet season to be one of its most conspicuous features; a regular, smaller, cool, dry-season epidemic has also been recognized [20]. In the present study there was an increase in the rate of isolation of intestinal parasites and of multiple pathogens during the summer wet season.

Enteric pathogens were identified in almost two-thirds of subjects with diarrhoea in this study and in 45% of symptomless children. There was a significant association between the presence of enteric pathogens and diarrhoea in infants under 2 years of age, while such organisms were found more commonly in symptomless children over 2 years of age. The very high rate of isolation of faecal pathogens in Aboriginal children said not to have diarrhoea may be because, in

our experience over many years, the stools of young Aborigines are nearly always loose or unformed. It is possible therefore that higher levels of endemic diarrhoeal disease exist than have been recognized [21]. We have previously shown that enterotoxigenic *E. coli* (ETEC) could be detected in 39% of Aboriginal children without recent or current diarrhoea and that isolations were much more frequent during the wet monsoonal summer than in the dry winter. Clearance of ETEC carriage occurred spontaneously always within 3 months [17].

In the present study isolations of bacterial or viral pathogens were significantly associated with diarrhoea, overall ($P \leq 0.025$), while isolates of bacteria, viral or multiple pathogens were significantly associated with diarrhoea in children under 2 years of age $(P \le 0.025)$. In children under 18 months of age mixed infections tended to be with viruses and bacteria while after that age mixed infections tended to involve intestinal parasites and bacteria. The most frequent bacterial infections were with enterotoxigenic and verotoxigenic $E.\ coli\ (ETEC\ and\ VTEC)$ and with Salmonella spp., Shigella spp. and Campylobacter spp. This is similar to our previously reported experience with more than 300 Aboriginal children hospitalized in Perth [2] and with 48 Aboriginal infants born in the Kimberlely region who were studied prospectively to 2 years of age [21]. The high rate of isolations of intestinal parasites in this study is also similar to the experiences accumulated by ourselves [2, 21, 22] and others [23-26] working with Aboriginal children in other parts of Australia. In the present study, Cryptosporidium was significantly associated with diarrhoeal episodes $(P \leq 0.01)$ while isolations of other intestinal parasites, including G. lamblia, were not; confirming our previous studies [2, 21]. G. lamblia is so highly endemic in children in Aboriginal communities that more than half can be infested at any one time [25, 26]. A recently reported survey from communities around Fitzroy Crossing, where the present work was undertaken, found G. lamblia in 32% of children's stools examined and H. nana in 16% and Entamoeba coli in 4% [27]; these are likely to be underestimates of the true prevalence of these parasites in this population. Aboriginal communities customarily have large numbers of domestic animals, particularly dogs, and it has been suggested that transmission from dogs and cats as well as human-to-human transmission of G. lamblia are involved in causing such high rates of giardiasis. This, in turn, has an important negative effect on the nutritional status of infants and children who live in these communities, often in heavily contaminated conditions.

Rotavirus was found in only a small proportion of children in the present study but was significantly associated with diarrhoea ($P \leq 0.01$), unlike our earlier experience with Aboriginal children in hospital in Perth in whom rotavirus was found just as frequently in children without diarrhoea as in those with diarrhoea [2].

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