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Virulence factors of enteric *Escherichia coli* in young Aboriginal children in north-west Australia

S. T. GUNZBURG[1], B. J. CHANG[1], V. BURKE[2] AND M. GRACEY[3]*

[1] *Department of Microbiology, University of Western Australia*
[2] *Department of Medicine, University of Western Australia, Royal Perth Hospital, Victoria Square, Perth, 6000, Australia*
[3] *Aboriginal Health Unit, Health Department of Western Australia, 189 Royal Street, East Perth, Western Australia, 6004*

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SUMMARY

Enterotoxigenic *Escherichia coli* (ETEC) were the most frequently identified enteric pathogens associated with diarrhoea in 0–5 year old Aboriginal children in tropical north-west Australia with an incidence similar to those from other tropical regions. Heat-stable toxin-producing (ST+) strains were associated with diarrhoea throughout the year but heat-labile toxin-producing (LT+) strains were more important in the monsoonal summer season. ST+ strains were commonest in children with diarrhoea between 6 and 18 months of age while LT+ strains were associated with diarrhoea in children aged 18–24 months. Verotoxigenic *E. coli* (VTEC) which produced VT1, but not VT2, and enteroadherent (EAF+) *E. coli* were significant causes of diarrhoea, mainly in children below 18 months but without a seasonal pattern.

INTRODUCTION

Gastrointestinal infections are prevalent and often severe in young Australian Aboriginal children [1] and are major contributors to their poor standards of health [2] and high rates of hospitalization [3]. Much of the published information about this subject comes from studies done in hospitals and little community-based work is available about the epidemiology of diarrhoea in young Aborigines and the causative microorganisms involved.

We have recently reported on the epidemiology of intestinal bacterial and viral pathogens and parasites in a prospective, year-long study of Aboriginal children in remote communities in tropical north-west Australia [4] and now report on the virulence mechanisms of *Escherichia coli* isolated from these children. The classes of pathogenic *E. coli* sought in this study included enteroadherent, enterohaemorrhagic, enteroinvasive, enteropathogenic, enterotoxigenic and verotoxigenic *E. coli*.

* Author for correspondence.

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METHODS

Subjects and samples

Faecal specimens were obtained from 104 Aboriginal children up to 5 years of age in the course of community health surveys in the early wet, late wet, early dry and late dry seasons in this tropical location (approximately 18° South) where the summer monsoon usually coincides with the diarrhoea season. Samples were collected by a community health nurse and Aboriginal health workers over 3 consecutive days. Specimens were placed into sterile, screw-topped plastic containers and inoculated into Cary-Blair transport medium, and transported overnight by air to Perth where they were cultured within 24 h.

Diarrhoea was defined as the passage of at least three 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 inspection of the stools.

Microbiological methods

Isolation and identification of *Escherichia coli* were performed as previously reported [4]. Five lactose-fermenting colonies, typical of *E. coli*, were isolated from each plate for further testing. All biochemically confirmed *E. coli* strains were blotted onto Zeta Probe membranes, each of which was hybridized with DNA probes to detect strains which were enterotoxigenic (heat-labile toxin = LT) [5], (heat-stable toxins = ST-1a and ST-1b) [6], verotoxigenic (VT1 and VT2) [7] and enteroadherent (EAF) [8]. The CVD 419 probe [9] was used to identify potentially enterohaemorrhagic *E. coli*: this probe was derived from a segment of a plasmid from an O157.H7 strain and hybridizes with most O157.H7 isolates and with many O26.H11 strains and other VT positive strains from patients with haemorrhagic colitis and haemolytic-uraemic syndrome [9]. Any lactose or non-lactose fermenting strain biochemically resembling an EIEC [10] was immediately tested for invasiveness in the Serény test [11]. *E. coli* strains that hybridized with EAF gene probes were tested for their ability to adhere to HEp-2 cells in a locally adherent pattern [12]. EAF^+ strains were also examined for their ability to accumulate actin in HEp-2 cells [13].

Statistical methods

Contingency tables were analysed using χ^2 tests or Fisher's exact test; a P value $\leqslant 0{\cdot}05$ was considered significant.

RESULTS

A total of 555 faecal specimens were examined, of which 173 were from children with diarrhoea. Approximately 35% of samples from children under 2 years of age were diarrhoeal specimens; in older children the proportion of diarrhoeal samples fell to 27% ($P \geqslant 0{\cdot}2$, n.s.). The isolation rates of enteric bacteria, viruses and parasites in this study has previously been reported [4]; enteric pathogens were identified in 65·3% of specimens from children with diarrhoea compared with 45·3% of samples from symptomless children. Enterotoxigenic *E. coli* (ETEC)

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Table 1. E. coli *isolated from Aboriginal children less than 5 years of age*

	No. of children (%)	
E. coli virulence factor	Diarrhoea	No diarrhoea
Enterotoxigenic	28 (16·2)	33 (8·6)*
ST	12 (6·9)	8 (2·1)*
LT	15 (8·7)	23 (6·0)
ST/LT	1 (0·6)	2 (0·5)
Verotoxigenic	26 (15·0)	23 (6·0)*
VT-1	13 (7·5)	8 (2·1)*
VT-2	10 (5·8)	15 (3·9)
VT-1/2	3 (1·7)	0 (0)
Enteroinvasive	0	0
Enteroadherent	14 (8·1)	14 (3·7)*
Total†	173 (100)	382 (100)

* $P < 0{\cdot}05$ for the difference between diarrhoeal and non-diarrhoeal samples.
† Total number of faecal samples examined.

Table 2. *Toxin production and seasonal associations*

	Wet season*		Dry season*	
Toxin	Diarrhoea†	No diarrhoea	Diarrhoea	No diarrhoea
ETEC	18 (20·7 %)‡	8 (6·1 %)	10 (11·6 %)	25 (10·0 %)
ST	8 (9·2 %)	5 (3·8 %)	4 (4·7 %)	3 (1·2 %)
LT	10 (11·5 %)‡	2 (1·5 %)	5 (5·8 %)	21 (8·4 %)
ST/LT	0	1 (0·8 %)	1 (1·1 %)	1 (0·4 %)
VTEC	11 (12·6 %)	9 (6·8 %)	15 (17·4 %)‡	16 (6·4 %)
VT-1	5 (5·7 %)‡	1 (0·8 %)	8 (9·3 %)‡	7 (2·8 %)
VT-2	5 (5·7 %)	6 (4·5 %)	5 (5·8 %)	9 (3·6 %)
VT-1/2	1 (1·2 %)	2 (1·5 %)	2 (2·3 %)	0
Total§	87 (100 %)	132 (100 %)	86 (100 %)	250 (100 %)

* Wet season, 1 November–30 April; dry season, 1 May–31 October.
† Number (%) of children found to carry a toxigenic strain.
‡ $P < 0{\cdot}05$ for the difference between diarrhoeal and non-diarrhoeal faecal samples.
§ Total number of faecal samples examined.

were the commonest bacterial pathogens associated with diarrhoea (16·2 % cf. 12·7 % for salmonella) (see Table 1). ST^+ strains were not associated with diarrhoea in the wet or dry season although significantly more children (with or without diarrhoea) were found to carry ST^+ strains during the wet season ($P < 0{\cdot}02$) (Table 2). LT^+ strains were associated with diarrhoea during the summer wet season ($P \leqslant 0{\cdot}005$) but not in the dry season which occurs in the milder winter months (Table 2). ST^+ strains were significantly associated with diarrhoea only in children aged between 6 and 18 months ($P < 0{\cdot}005$); LT^+ strains were significantly associated with diarrhoea only in the 18–24 month age group ($P < 0{\cdot}005$). Only three children were found to carry ST^+ and LT^+ strains.

Overall, VTEC infection was significantly associated with diarrhoea ($P \leqslant 0{\cdot}01$) (Table 1). All the VTEC strains isolated fermented sorbitol and failed to agglutinate with antiserum specific for *E. coli* strains belonging to the O157.H7 serotype. Furthermore, none of the VTEC strains hybridized with the CVD419

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gene probe. VTEC strains were significantly associated with diarrhoea in children less than 18 months of age ($P \leqslant 0{\cdot}001$), however there was no association of VTEC infection and diarrhoea in older children ($P \geqslant 0{\cdot}6$). Overall, VTEC infection was significantly associated with diarrhoea during the dry season ($P \leqslant 0{\cdot}002$), but not during the wet season ($P > 0{\cdot}05$) (Table 2).

VT1 was significantly associated with diarrhoea ($P \leqslant 0{\cdot}002$) while VT2 was not ($P \geqslant 0{\cdot}3$) (Table 1). Sixteen children less than 18 months of age, of whom 11 had diarrhoea, carried a VT1$^+$ strain. Only 5 older children were found to carry a VT1$^+$ strain and 3 of these children were symptomless at the time of sampling. All 8 children older than 18 months who were infected with a VT2$^+$ strain did not have diarrhoea at the time of sampling. Only 10 of the 17 children less than 18 months who were carrying a VT2$^+$ strain had diarrhoea at the time of sampling; and VT2$^+$ strains were not associated with diarrhoea in this age group ($P > 0{\cdot}5$). Three children with diarrhoea were found to carry both VT1$^+$ and VT2$^+$ strains. VT1$^+$ strains were associated with diarrhoea in both the wet ($P \leqslant 0{\cdot}03$) and dry ($P \leqslant 0{\cdot}015$) seasons. However, VT2$^+$ strains were not associated with diarrhoea in either season ($P \geqslant 0{\cdot}3$) (Table 2).

No blood or mucus was observed in any of the faecal samples. All lactose-positive *E. coli* strains were examined biochemically for the inability to decarboxylate lysine as a marker of enteroinvasive *E. coli* [10]. Strains that failed to decarboxylate lysine and all lactose-negative *E. coli* strains were tested in the Serény test. No enteroinvasive *E. coli* strains were identified.

Enteroadherent (EAF$^+$) *E. coli* carriage was a significant cause of diarrhoea particularly in children less than 18 months of age ($P \leqslant 0{\cdot}05$), however, there was no association with EAF$^+$ *E. coli* infection and diarrhoea in older children ($P \geqslant 0{\cdot}6$). There was a significant increase in the overall isolation rate of EAF$^+$ strains during the late dry and early wet seasons ($P < 0{\cdot}025$). EAF$^+$ carriage was associated with diarrhoea during this period of the year ($P < 0{\cdot}0015$).

Only one child with diarrhoea and two without diarrhoea carried EAF$^+$ strains that did not result in the accumulation of actin at the site of bacterial adherence to HEp-2 cells; actin-accumulating EAF$^+$ strains were significantly associated with diarrhoea ($P \leqslant 0{\cdot}025$). All EAF$^+$ strains that were negative in the actin accumulation test did not adhere to HEp-2 cells. All EAF$^+$ strains produced LA to HEp-2 cells, even those strains that did not cause actin accumulation.

DISCUSSION

ETEC were the bacterial enteric pathogens most frequently identified in faecal samples from young Aboriginal children who live in remote communities in the tropics in north-west Australia [4]. The overall isolation rate of ETEC was similar to those reported from tropical developing countries [14–16]. We found a significant seasonal variation, confirming the findings of a previous study in the same region [17] and similar to previous reports from Ethiopia [18] and Brazil [19]. A seasonal increase in *E. coli* contamination of stored water has been observed in the hotter months in Peru [20] but this is not a likely factor in our studies as water is not stored in this way in Aboriginal communities which are serviced with piped water from deep underground sources. The seasonal increase

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of *E. coli* infections in north-west Australia coincides with the summer monsoon and may be related to contamination of surface waters and increased survival and transmission of *E. coli* from warm, moist, contaminated soil and water, especially in toddlers and young children.

Overall, ST^+ strains were associated with diarrhoea but, with LT^+ strains, this occurred only during the wet season when their seasonality resembled a minor epidemic. This is in contrast with previous studies which found no association of LT^+ strains with diarrhoea [15, 16].

No children in the present study had symptoms of invasive, bloody diarrhoea which is typically associated with *E. coli* O157.H7 [21]. Apart from work on enterohaemorrhagic disease, there are few students on the clinical significance of verotoxigenic strains of *E. coli*. VTEC strains have been detected in symptomless Brazilian infants with diarrhea below 12 months [22] and in the present study a significant association with diarrhoea held only in subjects less than 18 months old. The rate of isolation of VTEC in our study was much higher than in a report from Thailand [23], perhaps because of geographical differences and methods of transmission and, perhaps, because of the presence of cattle in north-west Australia which are considered to be a major reservoir for VTEC infection of humans [24]. The lack of association of VTEC infection with diarrhoea during the wet season in the present study might be due to the high rate of symptomless carriage of $VT2^+$ strains during the wet monsoon. VT1 was the only verotoxin to be significantly associated with diarrhoea. $VT2^+$ strains, although being found somewhat more frequently than $VT1^+$ strains, were not associated with diarrhoea, perhaps because of the reported lower cytotoxic activity of VT2 [25].

EAF^+ *E. coli* were identified by the EAF gene probe and were found to be significantly associated with diarrhoea. All but three EAF^+ *E. coli* carriers were found to have strains which caused accumulation of actin in HEp-2 cells and these strains, therefore, possibly belong to EPEC serogroups [13]. The EAF probe detects genes conferring localised adherence of enteropathogenic *E. coli* to HEp-2 cells [8]. Localized adherence is also found among non-EPEC serotypes [26] and not all EPEC strains possess EAF plasmids [8]. However, most classical EPEC strains do cause actin accumulation in HEp-2 cells [13]. Routine EPEC detection may in future be facilitated by use of the *eae* gene probe which is specific for EPEC and EHEC strains demonstrating actin accumulation at the site of attaching and effacing lesions in tissue culture cells and *in vivo* [27].

EAF^+ EPEC strains have previously been shown to be associated with diarrhoea [28] although non-EPEC EAF^+ strains have been reported to not be associated with diarrhoea [28, 29]. This was not so in Thailand where EAF^+ non-EPEC strains were a significant cause of diarrhoea in infants below 6 months of age [28]. In the present study EAF^+ strains were a significant cause of diarrhoea only in children younger than 18 months. Other studies of children mainly below 12 months of age have associated EAF^+ *E. coli* strains with diarrhoea [28, 29] although their role in older children is uncertain. A gradual decline in the isolation of EPEC strains with increasing age has been observed in Mexican children [15], perhaps because of acquisition of immunological protection against reinfection later in life. A similar decline in isolation of EAF^+ strains occurred in the present study. The increased isolation rate of EAF^+ strains during the late dry and early

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wet seasons in our study is similar to observations made in Brazil [31] and to seasonal increases in EPEC-associated diarrhoea in South Africa which correlated with increased temperature rather than precipitation [32].

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