SHORT ARTICLE

A novel serotype of enteropathogenic *Escherichia coli* (EPEC) as a major pathogen in an outbreak of infantile diarrhoea

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An outbreak of infantile diarrhoea was investigated in 32 children, all <2 years old, in the tropical north of Australia. Rotavirus (63%) and enteropathogenic *Escherichia coli* (EPEC) (59%) were the most common pathogens identified. Of the 19 EPEC isolates, 14 (74%) were of serotype O126:H12, hitherto unreported as an EPEC serotype. Other pathogens isolated included *Salmonella* spp. (16%), *Campylobacter* spp. (3%), *Giardia* (3%) and *Shigella* spp. (3%). EPEC-related gastro-enteritis is an uncommon but recognised cause of diarrhoeal outbreaks in Australia and clinicians need to be aware of the possibility of this serotype being implicated. This report highlights the disadvantages of relying on serotyping alone for the recognition of EPEC.

Introduction

Since enteropathogenic *Escherichia coli* (EPEC) strains were first recognised as pathogens >50 years ago, their involvement as a major cause of infantile diarrhoeal outbreaks throughout the world has been well documented [1]. EPEC-associated diarrhoeal outbreaks in developed nations were common in the past but EPEC do not appear to be important in these countries now. There have been few reports of EPEC-associated diarrhoeal disease in Australia over the last 20 years [2].

Traditionally, EPEC have been shown to belong to a number of distinct serogroups and were once defined solely on the basis of their serotype [1]. However, with increasing understanding of EPEC, the possession of specific O and H antigens is no longer considered an essential characteristic [3]. The Second International Symposium on EPEC in 1995 determined that a 'typical EPEC' should have the ability to produce the

attaching/effacing (A/E) lesion, lack Shiga toxin and possess the EPEC adherence factor (EAF) plasmid [4].

This report describes a diarrhoeal outbreak in an isolated Australian Aboriginal community that was associated with a high prevalence of EPEC of a novel serotype.

Materials and methods

Study area and population

A total of 32 children <2 years old was affected; all resided in a small isolated Aboriginal community near Townsville, North Queensland, Australia. Climatic conditions in this region are generally hot and humid with mean temperatures of 33°C in January and 19°C in August. The wet season usually occurs from late November to the end of March.

Sample collection and preparation

Faecal samples collected at the local health facility in the community were sent to the Queensland Health Pathology Services Laboratory at Townsville General Hospital for preliminary microbiological testing. Samples were cultured for *Shigella*, *Salmonella*, *Aeromonas*, *Yersinia* and *Campylobacter* spp. by standard microbiological methods. Samples were also screened for rotavirus (Vidas, bioMérieux, France).

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Bacteria

E. coli control strains included two EAF-positive strains (E990, C771) and one EAF-negative strain (E611), obtained from the National *Escherichia coli* Reference Laboratory, Victorian Infectious Diseases Reference Laboratory, Melbourne, Australia.

Five lactose-fermenting colonies of non-mucoid appearance were selected from each MacConkey Agar plate (Becton-Dickinson, Microbiology Systems, Cockeysville, USA) and subcultured on to Nutrient Agar (MicroDiagnostics, Queensland, Australia). The resultant colonies were screened by slide agglutination with OK polyvalent antiserum (Murex Diagnostics, Dartford). All isolates that agglutinated with the polyvalent antiserum were then serotyped in tests with all standard O antisera from O1 to O173 and all standard H antisera from H1 to H56 [5].

PCR

Isolates were examined for the presence of the EPEC adherence factor (EAF) plasmid [6], the bundle-forming pilus (BFP) gene [7] and Shiga toxins 1 and 2 [8] by PCR as described previously.

HeLa cell adherence assay

HeLa cell adherence patterns were assessed by the method described by Scaletsky *et al.* [9].

Results

One or more of the recognised aetiological agents of diarrhoea were found in 30 (94%) patients. Multiple infection with *E. coli* and rotavirus occurred in 50% of the patients. Rotavirus was the most frequently identified pathogen, accounting for 63% of the cases, followed by EPEC (59%), *Salmonella* spp. (16%), *Campylobacter* spp. (3%), *Giardia* (3%) and *Shigella* spp. (3%). Only 12% of the patients had rotavirus as the sole pathogen. As the salmonellae isolated were of different serotypes, the cases were considered to be unrelated to each other.

Results of HeLa cell adhesion and PCR assays and serotyping (Table 1) showed excellent agreement between the genotypic and phenotypic assays. Of 21 *E. coli* isolates examined, 19 showed localised adherence (LA) in the 3-h assay and possessed the EAF plasmid and the BFP gene. All isolates were negative when tested for Shiga toxins 1 and 2. Of the six serotypes, O126:H12 was the most common (74%) of the EPEC isolates. We believe this to be a novel EPEC serotype as it has not been reported previously.

Table 1. Characteristics of 21 isolates of *E. coli* identified in this study

Serotype (Number of isolates if >1)	Adherence pattern	PCR detection of		
		EAF	BFP	Stx 1 and 2
O126:H12 (14)	LA	+	+	—
O55:H—	LA	+	+	—
O69:H27	LA	+	+	—
O99:H6	LA	+	+	—
O119:H2	LA	+	+	—
Ont:H10*	LA	+	+	—
O11:H55	NA	—	—	—
O107:H27	NA	—	—	—

LA, localised adherence; NA, no adherence pattern observed; EAF, EPEC adherence factor plasmid; BFP, bundle-forming pilus gene; Stx 1 and 2, Shiga toxins 1 and 2.

*O antigen not identified with O antisera O1–173.

Discussion

EPEC infections exhibit seasonality and are associated with warm and wet weather [10]. This outbreak occurred in mid-winter, which is usually very dry in this region of tropical Australia. However, there was a longer than normal wet season which extended to the end of May. The wet season was accompanied by cyclonic conditions that gave rise to extensive flooding. Furthermore, there is little seasonal variation in temperature in this region, with average maximum temperatures in summer and winter differing by as little as 6°C. These factors may explain the timing of this outbreak.

EPEC-associated diarrhoea is thought to be uncommon in developed countries and laboratories may no longer routinely screen for EPEC. Periodically, however, conditions may exist which mimic those in developing countries where outbreaks of EPEC are common. Thus, EPEC should not be discounted. Furthermore, this finding of a novel EPEC serotype (O126:H12) suggests that serotyping alone of *E. coli* isolates obtained from diarrhoeal outbreaks is useful but not definitive. This study highlights the need to use valid molecular methods for the detection of EPEC-associated virulence determinants.

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