J. Hyg., Camb. (1983), 90, 99–106
Printed in Great Britain

Studies on enterotoxigenic *Escherichia coli* isolated from persons without diarrhoea in Western Australia

By R. J. BERRY,*‡ K. A. BETTELHEIM† and M. GRACEY*

* *Princess Margaret Children's Medical Research Foundation, Perth, Western Australia, and* † *The National Health Institute, Wellington, New Zealand*

(*Received* 19 *August* 1982; *accepted* 16 *September* 1982)

SUMMARY

The epidemiology of enterotoxigenic *Escherichia coli* (ETEC) was studied in children without diarrhoea in two remote Aboriginal communities in tropical north-western Australia. Serial surveys of the same individuals during different seasons showed that isolations were much more frequent in the wet monsoonal summer than in the dry winter. All *E. coli* were isolated from symptomless children aged 5 years or less; in addition, clearance of ETEC carriage without treatment was observed in all individuals within 3 months of isolation. Of the 58 ETEC strains isolated, 40 had either an H32 or an O126 antigen. Five O antigens which have never been associated with ETEC (O2, O41, O71, O77 and O157) were found. A recently proposed system to detect ETEC, using groups of polyvalent antisera, would have detected only 3 out of these 58 ETEC strains.

INTRODUCTION

Gastrointestinal infections with enterotoxigenic *Escherichia coli* (ETEC) are important causes of diarrhoeal disease especially in infants and young children (Guerrant *et al.* 1975). However, the epidemiology of ETEC is poorly understood. Many studies report on collections of ETEC from diverse sources (Brunton *et al.* 1980; Deboy, Wachsmuth & Davis, 1980; Orskov *et al.* 1976; Reis *et al.* 1980). Other studies are based in hospitals (Merson *et al.* 1979). Searches for ETEC in the environment have largely been unsuccessful (Echeverria *et al.* 1978*a*; Echeverria *et al.* 1978*b*) although some recent surveys have identified ETEC in environmental sources including faeces from healthy people and cattle (Bettelheim *et al.* 1980; Bettelheim & Wilson, 1982).

Some recent studies have investigated community-based populations, both hospitalized (Merson *et al.* 1980*a*) and non-hospitalized patients and controls (Spencer *et al.* 1980). These infections are difficult to diagnose because ETEC are indistinguishable from non-enterotoxigenic *E. coli* (non-ETEC) by the routine serological methods normally used in clinical laboratories where techniques used for indentification of enterotoxins are not generally available. (Giannella, 1976; Yolken *et al.* 1977). This is especially so in less developed countries where ETEC infections are major causes of diarrhoea (Merson *et al.* 1980*a*).

‡ Present address: Epidemic Intelligence Service, Centers for Disease Control, Atlanta, Georgia, USA.

It has recently been proposed that most ETEC could be identified more simply by serogrouping, using polyvalent antisera selected from a range of serogroups and serotypes corresponding with heat labile toxin (LT) and heat stable toxin (ST) producing *E. coli* from several geographical locations (Merson *et al.* 1980*b*). Those authors called for further studies in other parts of the world to assess the place of antisera in diagnosis of ETEC diarrhoea and to determine whether modification of the constituent O groups proposed might improve the sensitivity of the approach.

We report results of toxin testing and serotyping of *E. coli* from Aboriginal children without diarrhoea from two remote communities in the tropical north of Western Australia.

MATERIALS AND METHODS

The subjects

These were 65 children from two remote Aboriginal communities, each of about 300 persons, in the Kimberley Region of the far north of Western Australia. They are several hundred kilometres apart and located at approximately 124° E, 17° S (Community 1) and 127° E, 14° S (Community 2) in a monsoonal tropical region with distinct wet (summer) and dry (winter) seasons. Surveys were done during both seasons. Both communities have staffed health clinics which the residents use regularly, and there were no reported outbreaks of diarrhoea prior to or during the surveys.

The specimens

Faecal specimens were collected and immediately inoculated into Carey–Blair medium without agar (Oxoid) and transported to Perth by air for testing. Representative strains of *E. coli.* were picked and stored in maintenance medium (Burke *et al.* 1981) until toxin testing was done.

Enterotoxin testing

The strains were tested for the production of heat-labile toxin (LT) using an enzyme-linked immunosorbent assay (Yolken *et al.* 1977) and heat-stable toxin (ST) with the suckling mouse assay (Giannella, 1976).

Serotyping

All strains were serotyped with *E. coli* O antisera (O1-O166) and H antisera (H1-H56) by previously described methods (Chandler and Bettelheim, 1974; Meckin, Bettelheim & Bacon, 1979).

RESULTS

Table 1 shows the community distribution of O serotypes and the number of different H antigens associated with each O serotype. In addition, it shows the number of strains isolated and the number of these which produced enterotoxin. The two communities had very different distributions of O serotypes with only O7, O8, and O153 common to both places. ETEC were only isolated from Community 1. There was also a seasonal variation in the number and distribution of O serotypes within each community (Table 2). During the wet season in Community 1 there was a greater diversity of O serotypes of non-ETEC than at other times of the year.

Table 1. *O serotypes, H antigens and enterotoxin production of* E. coli *isolated in Western Australia*

O serotype	No. of different H antigens	No. of strains isolated	Strains producing LT	Strains producing ST
	Community 1			
O2	3	7	5	—
O3	3	4	—	—
O4	1	2	—	—
O7	3	5	—	—
O8*	3	3	—	1
O9	1	1	—	—
O16	5	7	—	—
O17	1	1	—	—
O20*	2	2	—	—
O23	1	1	—	—
O25*	8	9	2	—
O33	2	2	—	—
O41	2	9	8	—
O48	1	1	—	—
O64	2	2	—	—
O68	1	1	—	—
O70	3	6	1	1
O77	5	7	3	—
O78*	3	4	—	—
O82	1	1	—	—
O88	2	3	—	—
O89	1	3	—	—
O91	1	1	—	—
O96	2	2	—	—
O111†	2	2	—	—
O126†	5	25	1	21
O152	1	1	—	—
O153	4	5	—	—
O157	2	2	1	—
O(29B2/3)	1	1	—	—
Ont	17	63	10	2
OR	2	4	—	2
Total	28	187	31	27
	Community 2			
O7	2	2		
O8*	2	2		
O36	1	1		
O48	2	3		
O69	2	2		
O89	2	2		
O93	1	1		
O106	1	1		
O141	1	1		
O153	1	1		
O163	1	1		
O166	1	1		
Ont	9	17		
Total	15	35	0	0

* These might be identified by the polyvalent antisera used by Merson *et al.* (1980).

† These are O antigens generally considered enteropathogenic. O(29B2/3) is an O antigen not yet accepted in the international scheme. Ont, not typable with standard sera O1 to O166. OR, rough.

https://doi.org/10.1017/S0022172400063890 Published online by Cambridge University Press

Table 2. *Seasonal distribution of O antigens of non-toxigenic* E. coli *strains in Western Australia*

	Community			Community 2	
Date...	October (1979)	February (1980)	May (1980)	August (1979)	February (1980)
Season...	Late dry	Wet	Early dry	Dry	Wet
	3	3	2	7	7
	7	7	4	36	8
	33	9	8	69	48
	68	20	16	93	89
	77	23	17	106	153
	111	25	25	141	163
	126	41	33	nt	166
	152	48	70	—	nt
	153	64	77		
	157	70	88		
	nt	78	96		
	—	82	126		
	—	89	153		
	—	91	nt		
	—	111			
	—	126			
	—	156			
	—	(29B2/3)*			
	—	R			
	—	nt			

nt, Not typable with standard sera O1 to O166.
R, Rough.
* An O antigen not yet accepted in the international scheme.

We isolated 31 LT+ and 27 ST+ toxin producers. Ten *E. coli* serotypes were found always to produce LT (Table 3(*a*)). However, 6 of 9 ST+ serotypes were not exclusively toxigenic because other non-toxigenic isolates were found with these serotypes (Table 3(*b*)). We isolated no LT+/ST+ *E. coli* in this study.

Of the 58 toxigenic strains isolated, 40 had either an H32 or an O126 antigen (Table 4). H32 was associated only with LT, and with one exception, O126 strains produced only ST. Two houses (11 and 28) had both antigens present at the same time, but not in the same child. Non-toxigenic O126 serotypes were present in house 11 during all three surveys; in addition, LT+ and ST+ O126 strains were both isolated during the wet season. A toxigenic *E. coli* (O126.HR) was isolated from one of five dogs tested.

Twenty of the 51 children in Community 1 age 5 years or less had ETEC isolated from their stools (Table 5). Only two children yielded LT+ ETEC in the dry season of late 1979; most LT+ ETEC and all ST+ ETEC were isolated from 18 children during the wet season in February 1980. LT+ ETEC were isolated from 12 children in 7 houses and ST+ ETEC were isolated from 8 children in 5 houses.

No child had both LT+ ETEC and ST+ ETEC at the same time, but 12 of 20 children had more than one toxigenic serotype present at the time of testing.

Table 3a. E. coli *serotypes from Community* 1 *which always produced enterotoxin*

Heat-labile enterotoxin		Heat-stable enterotoxin	
Serotype	No. isolated	Serotype	No. isolated
O2.H–	4	O8.H11	1
O25.H2	1	O126.H11	1
O25.H49	1	O126.H33	1
O41.H32	8		
O70.H27	1		
O77.H6	1		
O77.H32	1		
O126.H27	1		
O157.H–	1		
Ont.H32	8		
Total	27	Total	3

Ont, Not typable with standard sera O1 to O166; H–, non-motile.

Table 3b. E. coli *serotypes from Community* 1 *which did not always produce enterotoxin*

Heat-labile enterotoxin			Heat-stable enterotoxin		
Serotypes	No. isolated	No. toxigenic	Serotype	No. isolated	No. toxigenic
O2.H32	2	1	O70.H–	3	1
O77.H–	2	1	O126.H–	6	5
Ont.H7	9	1	O126.HR	16	14
Ont.H8	3	1	OR.H–	3	2
			Ont.H18	3	1
			Ont.HR	2	1
Total	16	4	Total	33	24

Ont, Not typable with standard O1 to O166; OR, rough; HR, rough; H–, Non-motile.

In patients 2 and 10, toxigenic and non-toxigenic strains of the same serotype were present. Pure cultures were uncommon, but patients 3 and 5 in house 6 had only ST+ O126.HR present.

DISCUSSION

This study of faecal *E. coli* from infants and young children in the far northwest of Australia has documented previously unknown toxigenic serotypes, an unusually high rate of asymptomatic carriage of ETEC, and spontaneous clearance of toxigenic organisms without treatment. It has also documented different distributions of *E. coli* serotypes between communities and within communities over time.

Our ETEC possessed five O antigens which have never been associated with toxin production (O2, O41, O70, O77 and O157) (Brunton *et al.* 1980; Deboy, Wachsmuth & Davis, 1980; Reis *et al.* 1980; Merson *et al.* 1979; Orskov *et al.* 1976; Spencer *et al.* 1980). This suggests that the combinations used in the polyvalent antisera proposed by Merson *et al.* (1980*b*) are not likely to detect LT+ or ST+ ETEC from children in the area studied, Only 5% (3/58) of our ETEC would have been detected

Table 4. *Distribution of H32 and O126 antigens within houses in Community 1*

	H32		O126		
				Toxins	
House	Strains	LT Toxin	Strains	ST	LT
6			16	15	
11	3	2	7	4	1
15	1	1			
16	10	8			
19	4	4			
28	3	3	2	2	
Total	21	18	Total 25	21	1

using their proposed method. In Community 1, 39% (20/51) of the children yielded ETEC without recent or current diarrhoea. The highest previously recorded isolation rate from asymptomatic individuals is 13·4% (Spencer *et al.* 1980). In addition, 3 months after the highest prevalence of ETEC, a survey which included all the children who had been toxin-positive, failed to find any ETEC. They had cleared spontaneously without treatment.

Non-toxigenic *E. coli* strains with the same serotype as LT-producers were not found frequently, but non-toxigenic strains with the same serotype as ST-producers were found commonly (Table 3*a*, *b*). This suggests that the ability to produce LT is more stable and lost less frequently than that of ST. Indeed, it is possible that the non-toxigenic O126 which was present in house 11 at the time of the first survey acquired the ability to produce ST and subsequently lost it.

Most studies indicate that so-called enteropathogenic serotypes, such as O126, do not normally produce enterotoxins (Scotland *et al.* 1981), however an outbreak of diarrhoea in adults and children has recently been described in New Zealand due to O126 strains producing ST (Bettelheim & Reeve, 1982). The O126 strains found in the present study which produced ST and LT are, therefore, highly unusual.

The distribution of the ETEC within the different subjects as shown in Table 5, demonstrates the great variability of *E. coli* serotypes. For example, patient 2 had two serotypes of O126, with H11 antigen or degraded H antigen, and enterotoxigen or not. It is unlikely to be coincidence that patient 9 had four different LT-producing serotypes all related antigenically. This seems to apply in most cases listed in Table 6, and this type of serological relatedness may explain the variety of serotypes shown in Table 1.

This study confirms the recently reported association between tropical wet seasons and ETEC isolations (Black *et al.* 1980), as well as confirming the geographical variation in *E. coli* serotypes, which had been suggested for some time. It supports the call by Merson *et al.* (1980) for further studies in other parts of the world to assess the place of antisera in diagnosis of ETEC diarrhoea. However, the lack of association between their proposed scheme and the pattern of *E. coli* isolation found in this study indicates a need for caution until more systematic data are available from different regions about the relationships between serological and toxigenic patterns of *E. coli*.

Table 5. *Isolation of enterotoxigenic* E. coli *from individuals in Community* 1 *in Western Australia*

	House	Subjects	Serotype	No. isolated	No. producing LT	No. producing ST
February 1980 ('wet' season)	2	1	O70.H27	1	1	
	6	2	O126.HR	4		3
			O126.H11	1		1
		3	O126.HR	6		6
		4	OR.H–	1		1
			O126.HR	1		1
			Ont.HR	1		1
		5	O126.HR	4		4
	9	6	O8.H11	1		1
	11	7	O126.H33	1		1
			O126.H–	3		3
			OR.H–	1		1
		8	O157.H–	1	1	
			O126.H27	1	1	
		9	Ont.H32	1	1	
			O77.H–	1	1	
			O77.H32	1	1	
			O77.H6	1	1	
	15	10	Ont.H18	2		1
		11	O2.H32	1	1	
			O2.H–	2	2	
	16	12	Ont.H32	2	2	
			O41.H32	3	3	
		13	O41.H32	2	2	
			Ont.H32	1	1	
		14	Ont.H8	1	1	
	19	15	O41.H32	1	1	
			Ont.H32	1	1	
		16	O41.H32	2	2	
	28	17	Ont.H32	3	3	
		18	O126.H–	2		2
			O70.H–	1		1
October 1979 ('dry' season)	38	19	O25.H49	1	1	
			O25.H2	1	1	
		20	O2.H–	2	2	
			Ont.H7	1	1	
Total				60	31	27

Ont, Not typable with standard sera O1 to O166; OR, rough; HR, rough; H–, non-motile.

ACKNOWLEDGEMENTS

We thank the Commissioner of Public Health for permission to publish this report. We are grateful to staff of Community and Child Health Services for help with organizing the surveys and collecting the specimens. We thank Airlines of Western Australia and the Royal Flying Doctor Services of Australia for help with travel. This work was supported financially by the TVW Telethon Foundation for Medical Research, Perth.

REFERENCES

Bettelheim, K. A. & Reeve, K. G. (1982). An outbreak of gastroenteritis due to enteropathogenic *Escherichia coli*, which are also enterotoxigenic. *New Zealand Medical Journal* **95**, 215–216.

Bettelheim, K. A. & Wilson, M. W. (1982). Enterotoxigenicity of strains of *Escherichia coli* isolated from the faeces of healthy people and cattle. *Journal of Hygiene* **88**, 121–133.

Bettelheim, K. A., Wilson, M. W., Shooter, R. A. & O'Farrell, S. M. (1980). Studies on the enterotoxigenicity of environmental *Escherichia coli*, belonging to serotypes normally considered to be enterotoxigenic. *Journal of Hygiene* **84**, 411–414.

Black, R. E., Merson, R. H., Rahman, A. S. M M., Yunus, M., Alim, A. R. M. A., Huq, I., Yolken, R. H. & Curlin, G. T. (1980). A two year study of bacterial, viral and parasitic agents associated with diarrhoea in rural Bangladesh. *Journal of Infectious Diseases* **142**, 660–664.

Brunton, J. Hinde, D., Langston, C., Glass, R., Rowe, B. & Gurwith, M. (1980). Enterotoxigenic *Escherichia coli* in central Canada. *Journal of Clinical Microbiology* **11**, 343–348.

Burke, V., Robinson, J., Berry, R. J. & Gracey, M. (1981). Detection of enterotoxins of *Aeromonas hydrophila* by a suckling mouse test. *Journal of Medical Microbiology* **14**, 401–408.

Chandler, M. E. & Bettelheim, K. A. (1974). A rapid method of identifying *Escherichia coli* H antigens. *Zentralblatt fur Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene* (Abteilung I: Orig. A) **229**, 74–79.

Deboy, J. M., Wachsmuth, I. K. & Davis, B. R. (1980). Serotypes of enterotoxigenic *Escherichia coli* isolated in the United States. *Infection and Immunity* **29**, 361–368.

Echeverria, P., Lin, Y.-L., Lee, C.-H., Verhaert, L. & Ewing, R. (1978*a*). Search for enterotoxigenic *Escherichia coli* in the environment in Taipei, Taiwan. *Chinese Journal of Microbiology* **11**, 138–140.

Echeverria, P., Verhaert, L., Basaca-Sevilla, V., Banson, T., Cross, J., Orskov, F. & Orskov, I. (1978*a*). Search for enterotoxigenic *Escherichia coli* in humans, livestock, food, and water in a community in the Philippines. *Journal of Infections Diseases* **138**, 87–90.

Giannella, R. A. (1976). Suckling mouse model for detection of heat stable *Escherichia coli* enterotoxin: charactistics of the model. *Infection and Immunity* **14**, 95–99.

Guerrant, R. L., Moore, R. A., Kirschenfield, B. A. & Sande, A. (1975). Role of toxigenic and invasive bacteria in acute diarrhea of childhood. *New England Journal of Medicine* **293**, 567–573.

Meekin, G. E., Bettelheim, K. A. & Bacon, D. F. (1979). Serotypes of antibiotic resistance *Escherichia coli* isolated from the sewage of Palmerston North (New Zealand). *Journal of Hygiene* **82**, 443–452.

Merson, M. H., Black, R. E., Khan, M. U. & Huq, I. (1980*a*). Epidemiology of cholera and enterotoxigenic *Escherichia coli* diarrhoea. In *Proceedings of the 43rd Nobel Symposium on Cholera and Related Diarrhoeas – Molecular Aspects of Global Health Problem*, pp. 34–35. Basel: S. Karger.

Merson, M. H., Orskov, F., Orskov, I., Sack, R. B., Huq, I. & Koster, F. T. (1979). Relationship between enterotoxin production and serotype in enterotoxigenic *Escherichia coli*. *Infection and Immunity* **23**, 325–329.

Merson, M. H., Rowe, B., Black, R. E., Huq, I., Glass, R. I. & Eusof, A. (1980*b*). Use of antisera for identification of enterotoxigenic *Escherichia coli*. *Lancet* ii, 222–224.

Orskov, F., Orskov, I., Evans, D. J., Sack, R. B. & Wadstrom, T. (1976). Special *Escherichea coli* serotypes among enterotoxigenic strains from diarrhoea in adults and children. *Medical Microbiology and Immunology* **162**, 73–80.

Reis, M. H. L., Matos, D. P., Castro, A. F. P. D., Toledo, M. R. F. & Trabulsi, L. R. (1980). Relationship among enterotoxic phenotypes, serotypes, and sources of strains in enterotoxigenic *Escherichia coli*. *Infection and Immunity* **28**, 24–27.

Scotland, S. M., Day, H. P., Cravioto, A., Thomas, L. V. & Rowe, B. (1981). Production of heat-labile or heat-stable enterotoxins by strains of *Escherichia coli* belonging to serogroups of O44, O114 and O128. *Infection and Immunity* **31**, 500–503.

Spencer, H. C., Wells, J. G., Gary, G. W., Sondy, J., Puhr, N. D. & Feldman, R. A. (1980). Diarrhoea in a non-hospitalized rural Salvadoran population: the role of enterotoxigenic *Escherichia coli* and Rotavirus. *American Journal of Tropical Medicine and Hygiene* **29**, 246–253.

Yolken, R. H., Greenberg, H. B., Merson, M. R., Sack, R. B. & Kapikian, A. Z. (1977). Enzyme-linked immunosorbant assay for detection of *Escherichia coli* heat-labile enterotoxin. *Journal of Clinical Microbiology* **6**, 439–444.