The enterotoxigenicity of strains of *Escherichia coli* isolated from the faeces of healthy people and cattle

By K. A. BETTELHEIM AND M. W. WILSON

*National Health Institute, Department of Health, P.O. Box 7126,
Wellington South, New Zealand*

(Received 20 July 1981)

SUMMARY

In this study 197 strains of *Escherichia coli* isolated from cowpats and the faeces of healthy humans were examined for the presence of enterotoxins. Six strains representing five different serotypes were found to be enterotoxigenic. Four of these strains produced the heat labile enterotoxin and two, the Vero cell cytotoxin.

INTRODUCTION

In a recent study on 15 strains of *Escherichia coli*, which had been isolated from cowpats and meat (Bettelheim *et al.* 1976), two strains belonging to serogroups O8 were found to be enterotoxigenic. One of these strains produced the heat labile enterotoxin (LT) and the other was weakly positive for the heat stable enterotoxin (ST) (Bettelheim *et al.* 1980). The strains had been specifically chosen from the large collection of environmental *E. coli*, which had been collected (Bettelheim, 1978) and serotyped to represent those 'OH' serotypes which Orskov *et al.* (1976) found to be predominantly enterotoxigenic in their studies on serotypes of *E. coli* from human cases of diarrhoea.

It was therefore decided to investigate a larger collection of strains from various sources. In many of the studies from which these strains of *E. coli* were isolated, up to ten colonies per specimen were picked and so strains of the same or different serotype picked from the same specimen were examined. In this study further strains from cowpats collected in England and Wales were examined as well as strains isolated from the faeces of healthy humans.

MATERIALS AND METHODS

Bacterial Strains

The test strain for the LT and ST enterotoxin assays was *E. coli* H-10407 (078.H11). It had been obtained from Dr D. J. Evans Jr (University of Texas Medical School at Houston, Houston, Texas, U.S.A.).

The strains studied included 38 strains isolated from cowpats (Bettelheim *et al.* 1976), 90 strains from mothers and their healthy babies isolated during a study on the ecology of *E. coli* in a maternity ward in London (Bettelheim *et al.* 1974) and 69 from the faeces of a nurse in London isolated over an eight week period in a study on the effect of diet on intestinal *E. coli* (Bettelheim *et al.* 1977).

Table 1. *Strains of E. coli which were found to be enterotoxigenic*

Strain	Source	Serotype	Enterotoxin found
C22/1 } C22/9 }	Cowpat	{ 0109 . H21 { R . H21	LT LT
9M1/8 } 9M1/9 }	Healthy mother	{ 020 . H- { 020 . H-	VT VT
30M1/4	Healthy mother	0141 . H2	LT
SN2/f/5	Healthy nurse	R . H56	LT

Heat labile and heat stable enterotoxin assays

Similar standard methods as described before were used for the LT and ST assays (Bettelheim *et al.* 1980; Dean *et al.* 1972).

Cytotoxin Assay

The method of Konowalchuk *et al.* (1978) was used to assay for the production of the Vero cell cytotoxin (VT).

RESULTS

The strains which were found to be toxigenic in these studies and their serotypes are listed in Table 1. These results show that two pairs of toxigenic strains were isolated from one specimen each. It is likely that the rough strain (C22/9) is a degraded variant of C22/1. The other eight strains isolated from this cowpat belonged to other serotypes (0104 . H8 and 058 . H40). In the earlier study (Bettelheim *et al.* 1980), two strains from cowpat C72 were studied and one strain (08 . Hnt) was found to be enterotoxigenic. None of the other strains from this cowpat were found to be enterotoxigenic despite one being the same serotype. Although not listed in Table 1, strain C72/3 was still enterotoxigenic.

The specimen from mother 9, which yielded the two cytotoxic *E. coli*, contained two other strains of the same serotype but these were not enterotoxigenic. These serotypes were not transmitted to her baby.

Mother 30 from whom the enterotoxigenic serotype 0141 . H2 was isolated, yielded in that specimen three more isolates of the same serotype. Although this was the only serotype of the ten strains picked from the baby's first specimen, none of these strains were enterotoxigenic.

The only strain found to be enterotoxigenic of the types isolated from the nurse was isolated during the study period, during which the nurse was on a sterile diet.

None of the strains studied produced the ST.

DISCUSSION

Enterotoxigenic *E. coli* have in recent years been found to be involved in a number of outbreaks of gastroenteritis and individual cases. It is thus important to assess the distribution of these organisms in the environment. The faeces of healthy humans and cattle provide a suitable basis for such studies.

Recent studies by a number of authors have suggested enterotoxigenic strains of *E. coli* do not play a major role in temperate countries; however, they have been shown to be present in some cases of gastroenteritis. This study confirms that these organisms are perhaps more widespread than had been anticipated, particularly as six out of 197 strains or nearly 3% were toxigenic. As none of these caused disease it is suggested that these strains lacked some other virulence factors.

This paper is published with the authority of the Director-General of Health, Department of Health, Wellington, New Zealand.

REFERENCES

- BETTELHEIM, K. A. (1978). The sources of 'OH' serotypes of *Escherichia coli*. *Journal of Hygiene* **80**, 83-113.
- BETTELHEIM, K. A., BREADEN, A., FAIERS, M. C., O'FARRELL, S. M. & SHOOTER, R. A. (1974). The origin of 'O' serotypes of *Escherichia coli* in babies after normal delivery. *Journal of Hygiene* **72**, 67-70.
- BETTELHEIM, K. A., COOKE, E. M., O'FARRELL, S. & SHOOTER, R. A. (1977). The effect of diet on intestinal *Escherichia coli*. *Journal of Hygiene* **79**, 43-45.
- BETTELHEIM, K. A., ISMAIL, N., SHINEBAUM, R., SHOOTER, R. A., MOORHOUSE, E. & FARRELL, W. (1976). The distribution of serotypes of *Escherichia coli* in cowpats and other animal material compared with serotypes of *E. coli* isolated from human sources. *Journal of Hygiene* **76**, 403-406.
- 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 enterotoxigenic. *Journal of Hygiene* **84**, 411-414.
- DEAN, A. G., CHING, Y. C., WILLIAMS, R. G. & HARDEN, L. B. (1972). Test for *Escherichia coli* enterotoxin using infant mice: Application in a study of diarrhoea in children in Honolulu. *Journal of Infectious Diseases* **125**, 407-411.
- KONOWALCHUK, J., DICKIE, N., STAVRIC, S. & SPEIRS, J. I. (1978). Comparative studies of five heat labile toxic products of *Escherichia coli*. *Infection and Immunity* **22**, 644-648.
- ORSKOV, F., ORSKOV, I., EVANS, D. J. JR, SACK, R. B., SACK, D. A. & WADSTROM, T. (1976). Special *Escherichia coli* serotypes among enterotoxigenic strains from diarrhoea in adults and children. *Medical Microbiology and Immunology* **162**, 73-80.