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Enterotoxigenic enteric bacteria in foods and outbreaks of food-borne diseases in Sweden

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SUMMARY

All of 86 foods routinely examined for potentially pathogenic enteric bacteria were found to harbour one or more coliform species. None of the strains isolated produced heat-labile enterotoxin (LT) or showed invasive properties. The suckling mouse test indicated that one strain of *Escherichia coli* produced heat-stable enterotoxin (ST). Twelve incidents of suspected food poisoning were also investigated. In two of them the foods examined contained LT-producing strains of *E. coli* and in two there were LT-producing strains of *Klebsiella pneumoniae*. The counts of viable enterotoxigenic micro-organisms in these foods were 3000–30000 *E. coli*/g and 50000 to 1 million *K. pneumoniae*/g. The dominant symptom in all the incidents was watery diarrhoea. These seem to be the first reported cases of foodborne enterotoxigenic enteric bacteria in Europe. Though enterotoxigenic *E. coli* and related gram-negative enterotoxin-producing species are rare in correctly handled food in Sweden, these micro-organisms should be searched for when outbreaks of food poisoning are investigated.

INTRODUCTION

Enterotoxigenic *Escherichia coli* is a common cause of travellers' diarrhoea in many countries (Gorbach *et al.* 1975; Bäck, Blomberg & Wadström, 1977). Other enteric micro-organisms, such as *Klebsiella pneumoniae, Aeromonas hydrophila* and various *Proteus, Enterobacter* and *Serratia* species isolated from African children with choleriform diarrhoea (Wadström *et al.* 1976; Schoub *et al.* 1977) and from Swedish U.N. soldiers (Bäck, Jonsson & Wadström, 1978) have been found to produce extracellular enterotoxin.

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Table 1. *Foods investigated, not involved in food-poisoning incidents*

Type of food	Number of samples	Number of strains investigated
Swedish products		
Raw meat	11	58
Vegetables	9	36
Ready-to-eat food*	14	59
Dairy products	23	73
Imported products		
Raw products	7	38
Unpasteurized soft cheeses	22	69
Total	86	333

* Foods which can be consumed without further processing. The designation includes convenient dishes, sandwiches, meat salads.

Despite rapid progress in the understanding of cholera and *E. coli* enterotoxins, there are few reports on the frequency of enterotoxigenic *E. coli* in food and water (Mehlman *et al.* 1976). A recent survey in the U.S.A., however, showed that 8% of 240 *E. coli* strains isolated from food produced either heat-labile (LT) or heat-stable (ST) enterotoxin (Sack *et al.* 1977). The writers concluded that tests for *E. coli* in food should not be limited to determining if the isolates are 'enteropathogenic serotypes'.

The present study was undertaken in 1977 as a first attempt to evaluate the relative importance of enterotoxigenic *E. coli* and other gram-negative micro-organisms in foods on sale in Sweden and in outbreaks of food poisoning in which coliforms were the suspected agents.

MATERIALS AND METHODS

Sources of bacteria

From various food products not known to be involved in food poisoning, 333 strains of *E. coli* and other lactose-fermenting gram-negative micro-organisms were isolated. The types of food are listed in Table 1. The samples were collected in the Stockholm area between December 1976 and April 1977 and were examined at the Stockholm Health Board's Department of Bacteriology or at the College of Veterinary Medicine's Department of Food Hygiene.

Twelve foodstuffs suspected to have been the cause of diarrhoeal disease, with 'coli-like' symptoms were also investigated. These were salads containing shrimp and mushrooms, ham and crayfish tail, baked roast beef, home made liver sausage, cooked tongue, cooked meat, hot dogs, black pudding, mushrooms cooked in cream, boiled rice, home made cheeses and cream cakes.

Bacteriological examination

Complete bacteriological examination according to the regulations issued in 1977 by the Swedish National Food Administration was performed on all the specimens. The tests were essentially as described in *Microorganisms in Foods; their significance and methods of enumeration* (ed. Thatcher & Clark, 1968). Coliforms were isolated from violet red bile agar (Difco) plates incubated at 37 °C for 1 day and at 44 °C for 2 days, respectively. One to five typical colonies from each plate were subcultured on tryptose lactose agar supplemented with bromo-cresol purple. Each isolate was then biochemically identified with the API 20 E Profile Recognition Index (Analytab) according to the manufacturer's instructions (Nord, Wadström & Dahlbäck, 1975).

Testing for enterotoxin

All coliforms isolated were cultured 24 h in brain–heart infusion broth (Difco) or in tryptone yeast extract medium (TY). The extracellular fluid was immediately assayed for heat-labile enterotoxin (LT) using the adrenal Y1 cell test (Donta *et al.* 1974) or the Chinese hamster ovary cell (CHO) test (Guerrant *et al.* 1974) or both. One hundred and six of the strains from routine samples and all strains from suspected food-borne disease were also cultured in a casamino acids yeast extract medium (CAY; Evans & Evans, 1973) and tested for ST production in suckling mice according to Gianella (1976).

Test for invasiveness

Three hundred of the 333 strains from routinely collected food samples were cultured in nutrient broth (Difco) supplemented with 5% (v/v) glycerol. The samples were incubated on a rotary shaker for 18 h at 37 °C. One drop (0·05 ml) of this suspension was instilled into the lower conjunctival sac of a guinea-pig – the Serény keratoconjunctivitis test (Serény, 1957).

Serotyping

The enterotoxigenic strains of *E. coli* were examined by slide agglutination for O, K and H antigens, using a standard set of antisera. These tests were done at the WHO International Escherichia Reference Centre in Copenhagen (Ørskov *et al.* 1976).

RESULTS

Food specimens collected for routine bacteriological examination

All of the 86 specimens analysed (Table 1) contained coliform bacteria. The species are shown in Table 2. A diarrhoeic virulence factor was found in only one strain (Table 3). This was an *E. coli*, isolated from unpasteurized milk, which produced heat-stable enterotoxin (ST).

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Table 2. *Investigated coliform bacteria from foods not involved in food poisoning incidents*

Species/genera	Number of strains isolated	%
Enterobacter	124	37
Escherichia coli	116	35
Klebsiella	60	18
Citrobacter	20	6
Serratia	13	4
Total	333	100

Table 3. *Virulence factors in coliform bacteria isolated from foods not involved in food poisoning incidents*

Virulence factor	Positive	Negative
Heat-labile enterotoxin (LT)	0	333
Heat-stable enterotoxin (ST)	1	105
Invasive properties	0	300

Food-poisoning incidents

Incident 1. Shrimp and mushroom salad was served in a Swedish restaurant on two consecutive days in December 1976. Of the 106 persons who ate this salad, 60 (58 %) became ill after 12 h with severe, acute diarrhoea and abdominal pain. The maximum duration of the symptoms was 24 h. Three specimens from the salad were examined and each was found to contain about 3000 *E. coli*/g. *E. coli* strains from all three specimens were shown to produce heat-labile enterotoxin, confirmed both by ovary and adrenal cell tests. None of the tested strains produced heat-stable enterotoxin. The serotypes of the three LT-producing strains were O25:K2:H12, O22, IF1:H- and O11:K16:H4, respectively.

Incident 2. A consumer of cold, shop-sliced roast beef developed acute watery diarrhoea 19 h after the meal. The symptoms lasted for less than 24 h. The roast beef was found to contain about 30000 *E. coli* (O8:K44:H28) per g. Heat-labile enterotoxin was produced by this strain in both test systems, but not heat-stable enterotoxin.

Incident 3. About 7 h after eating ready-cooked tongue, a man and wife became ill with abdominal pain, diarrhoea and occasional vomiting. The symptoms lasted only about 5 h. The tongue contained about 50000 LT-producing *K. pneumoniae*/g, as shown by the adrenal Y1 and the CHO cell tests.

Incident 4. Consumption of cooked sausage (hot dogs) was followed after 5 h by violent diarrhoea, abdominal cramps, vomiting and slight pyrexia (*c.* 38 °C) in two persons. The symptoms subsided after 24 h. The sausage contained about 1 million *K. pneumoniae* per g. The reason for this high count may have been that before transport to the laboratory the sausages were stored overnight at room temperature in their cooking water, and then in a refrigerator for 2 days.

The *K. pneumoniae* strain isolated was LT-positive when tested in CHO cells, but negative in the adrenal Y1 cell test. No ST was detected.

Other incidents. From the foodstuffs in the remaining eight incidents of food poisoning, no enterotoxigenic strains were isolated, nor could any other bacteria commonly involved in foodborne diseases (staphylococci, salmonellas, shigellas, *Clostridium perfringens* or *Bacillus cereus*) be found.

DISCUSSION

Research on foods as vehicles of travellers' diarrhoea was begun fairly recently, with the discovery that the cause often was enterotoxigenic *E. coli* (Gorbach *et al.* 1975; Sack, 1975). Up to now there are few reports of enterotoxin-producing *E. coli* as the agent in food-borne outbreaks of diarrhoeal disease (Sack, 1978). In 1976 more than 2200 staff members and visitors to an American national park fell ill with gastro-intestinal symptoms. The drinking water was shown to be contaminated with sewage. Enterotoxigenic (LT) *E. coli* were recovered from the water supply and from the infected persons' stools (Rosenberg *et al.* 1977). Two other outbreaks of diarrhoeal illness were attributed to enterotoxigenic *E. coli* in food, but the association with specified foods was only statistically confirmed (CDC, 1976; Merson *et al.* 1976).

In our investigation, enterotoxigenic gram-negative bacteria were isolated from 4 of 12 foodstuffs suspected of being the cause of food poisoning. This seems to be the first documentation in Europe of enterotoxin-producing coliform bacteria as the causal factor in food-borne intoxication. Moreover, two of the enterotoxic strains recovered from food in separate poisoning incidents were *K. pneumoniae.*

Enterotoxigenic enteric micro-organisms other than *E. coli* have not previously been reported as a cause of food poisoning. But studies on acute diarrhoeal disease in Africa and on tropical sprue in Puerto Rico have shown that gram-negative enteric micro-organisms other than *E coli* can produce enterotoxin of both LT and ST type (Klipstein *et al.* 1973; Wadström *et al.* 1976; Schoub *et al.* 1977). Enterotoxigenic *Enterobacter* have been isolated from children in Poland (Wadström; personal communication) and from persons with travellers' diarrhoea in Cyprus (Bäck *et al.* 1978).

All the isolated *E. coli* strains in our food-poisoning cases were of different serotypes. Even the three strains in Incident 1 were serologically different. This finding has special interest in view of the fact that the ability to produce enterotoxin is determined by transmissible plasmids (Gyles, So & Falkow, 1974). Sack *et al.* (1977) similarly found two or three different serotypes of enterotoxigenic *E. coli* in the same faecal specimens. Other investigators (e.g. Ørskov *et al.* 1976; Scotland, Gross & Rowe, 1977) stated that the enterotoxigenic *E. coli* may be of a relatively limited number of serotypes. Since testing for enterotoxins with current methods is laborious, Scotland *et al.* (1977) suggested serologic prescreening followed by study of certain selected serotypes for enterotoxin production. Such a simplified procedure may be questionable, however, as 'new' enterotoxigenic serotypes could be missed.

In the foods examined in the reported incidents of food poisoning, the content of coliform bacteria ranged from 3000 to 1 millon per gram. These figures exceed the Swedish limits for acceptable bacteriological quality in food (< 10000 37 °C and < 1000 44 °C coliforms/g food). On the other hand, the numbers of *E. coli* detected were low in relation to the infective dose (10^9–10^{10}) reported by DuPont *et al.* (1971) from experiments in man. In a more recent study, however, Levine *et al.* (1977) showed that as few as 10^6 micro-organisms evoked diarrhoeal symptoms in 50% of experimental persons when the gastric juice was neutralized. The incubation time and the nature and duration of the symptoms in the food poisoning incidents here reported agree well with descriptions in the literature (Sack, 1975; CDC, 1976).

Enterotoxigenic *E. coli* and other enterotoxin-producing, lactose-fermenting enteric micro-organisms were found to be uncommon in Sweden in imported foods and in correctly handled and stored Swedish foods. The only enterotoxic strain found in the routinely collected, non-suspect samples was an ST-producing *E. coli* (Kudoh *et al.* 1977) isolated from unpasteurized milk. Enterotoxigenic *E. coli* has been associated with diarrhoeal disease in calves and pigs (Smith & Halls, 1967), and it seems possible that this strain originated from domestic animals. Enterotoxigenic gram-negative bacteria also seem to be an unusual cause of diarrhoea in Sweden, since most of such strains have been found in tourists returning from Mediterranean countries (Bäck *et al.* 1977).

Our study shows that, though enterotoxigenic gram-negative bacteria seem to be rare in correctly handled food, such micro-organisms should not be forgotten in investigations of food poisoning. Special attention should be directed to 'ready to eat' foods, since these have often been in close contact with the makers' hands and frequently are consumed without pre-heating.

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