Enterohaemorrhagic *Escherichia coli*: A new problem, an old group of organisms

Inter faeces et urinam nascimur

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SUMMARY: All mammals are colonised by *Escherichia coli* generally at birth and these organisms become part of their intestinal flora for the rest of their lives. New types are acquired generally by an oral route. Some *E coli* are pathogenic and some may have a far more enhanced ability to colonise the human intestine than most others. Recently enterohaemorrhagic *E coli* have emerged. They can cause a number of intestinal illnesses in humans including bloody diarrhoea and haemolytic uraemic syndrome. These organisms produce a number of virulence factors particularly the Shiga-like toxins (verotoxins). The intestines of animals may be the reservoir of these organisms for human infection, and cattle particularly have been shown to harbour them. Food, especially undercooked meat products, have been associated with a number of outbreaks throughout the world. While a certain serotype O157.H7 has been associated with many outbreaks throughout the world, other serotypes, particularly O111.H-, have also been reported. This latter serotype appears to be more common in Australia.
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Introduction

According to the Latin proverb, we are born between the urine and the faeces. Thus from birth we acquire the faecal flora of our mothers.

Over a century ago Escherich described the bacteria that he isolated from the faeces of human neonates as *Bacterium coli commune* (Bettelheim 1986; Escherich 1988). He demonstrated that the organisms now known as *Escherichia coli* were present in the faeces and intestinal contents of humans and were considered as commensal organisms. The biochemical tests that are the substance of modern bacterial taxonomy were developed over the ensuing decades and became the basis of the taxonomy of the enterobacteriaceae in general and *E coli* in particular. Kauffmann (1947) established the serotyping of *E coli*, which permitted the far greater understanding of the ecology of these bacteria. Besides being subdivided into serotypes, *E coli* can also be subdivided into biotypes by their antibiotic resistance patterns (Crichton and Old 1992) and isoenzyme patterns (Goullet and Picard 1986). The clonal nature of *E coli* has been specifically addressed (Achtman 1985; Whittam 1989). Although *E coli* are the most extensively studied organisms from the faeces of humans and animals, they comprise only a very small percentage of the total faecal flora. Mitsuoka and Hayakawa (1972) estimated the enterobacterial flora of human faeces to range from values of 10^8 to 10^9/g whereas the total bacterial flora was around 10^{10}/g.

Over the last 15 years a number of pathogenic groups of *E coli* that affect both humans and animals has been described, especially the enterohaemorrhagic *E coli* (EHEC). They have been associated with many cases and outbreaks of severe diarrhoea often including haemolytic uraemic syndrome and sometimes causing death. These EHEC occur normally in the gut of domestic animals and food may be the main vehicle of transmission to humans. This review examines the emergence of EHEC as pathogens to humans.

Neonatal Colonisation

Acquisition of E coli *by Domestic Animals*

Mammals acquire their *E coli* from their dams at birth (Smith and Crabbe 1956; Hinton *et al* 1985) and from the other animals. The diversity of the *E coli* of farm animals decreases as they get older (Hinton 1985). Smith (1971) found that during the first day of life of piglets, the alimentary tract is flooded with large numbers of *E coli* as well as other bacteria, while the pH of the stomach is initially high. As the piglets grow older, the acidity of their stomach increases and the numbers of all bacteria, except lactobacilli, decrease.

Acquisition of E coli *by Human Babies*

Initial studies on humans were directed towards gaining an understanding of the acquisition of enteropathogenic *E coli* (EPEC) by neonates. These EPEC were generally derived from the mother's genital tract (Ocklitz and Schmidt 1957). Many other studies indicated that EPEC can be transmitted from mothers to their infants at birth. While such EPEC serogroups could be isolated from healthy children, Gage *et al* (1961) showed that serogroups other than EPEC were commonly found in the faeces of healthy babies.

In an extensive study in the early 1970s, Bettelheim *et al* (1974a) collected many isolates of *E coli* from the faeces of mothers, their babies and the mucus extracted from the babies' mouths after delivery. Isolates from most mothers and their babies were of the same O group. Where *E coli* was cultured from the mucus, the isolates tended to comprise an intermediate distribution of O groups between the faecal cultures from the mother and her baby. The variety of O serogroups isolated from some but not all mother/baby pairs was greatest in maternal faeces, least in the baby's faeces and intermediate in the baby's mucus. By increasing the number of phenotypic characters of *E coli* examined (Bettelheim *et al* 1974b), the transmission of *E coli* during birth was further demonstrated. Some of the characteristics of the *E coli* were either lost or gained during such transmission, suggesting that there must be some environmental pressure on these organisms (O'Farrell and Bettelheim 1976; Shinebaum *et al* 1977).

In addition it was found that each baby was a focus for the spread of its *E coli* to other babies, in the neonatal ward, particularly to those born by Caesarean section. Similar conclusions were reached in another study (Graham and Taylor 1976). However, where the

mothers predominantly looked after their own babies, virtually no spread was observed between babies (Bettelheim *et al* 1983b). *E coli* acquision by the babies from their mothers was as prompt as in the previous studies.

Spread of Enteropathogenic E coli

While any *E coli* can spread from person to person, some pathogenic types appear far more able to do so. This was most strikingly demonstrated in the study of Bettelheim *et al* (1983a). In the same maternity ward there was a outbreak of gastroenteritis caused by an EPEC strain O125.H21. Despite full precautions, including closing the ward to new admissions and barrier nursing the babies, this EPEC strain spread to 16 babies. Other serotypes spread to only three babies. This spread did not differ from the spread observed under the normal conditions described above. One case even occurred 25 days after the index case in a two-year-old child in a separate ward block. The only connection was the medical staff.

This study demonstrates two significant points about the acquisition of *E coli* by babies. Despite precautions being taken to prevent the spread of the EPEC, its spread was not prevented and it demonstrated an ability to spread to other babies far greater than that observed for 'normal' *E coli*. The inability to prevent the spread of the 'normal' *E coli* suggests that even these have some ability to spread.

The Food Chain
Acquisition of Commensal E coli *by Adults*

In order to gain a greater understanding of the possible acquisition of non-pathogenic *E coli* by healthy adults, a group of individuals was observed for periods ranging from two to five months (Shooter *et al* 1977) and their faecal *E coli* serotyped. Some of these people yielded very few *E coli* serotypes. From one subject, only *E coli* O2.H6 was isolated throughout the three months' period of study. Three other subjects yielded three, four or seven different serotypes each. In one of these subjects seven of the 12 specimens examined contained exclusively *E coli* O22.H1 and the only other serotypes isolated from this person were ORough.H1 and O106.H1.

Although there was a greater variety of serotypes in the other subjects, all showed that one serotype must be a persistent type. The person with the greatest variety of serotypes ate in canteens. It was found that of the only married couple in this series both individuals carried the same serotype O1.H7. The variety of serotypes carried by the wife was lower (seven) compared with that of the husband (21) although an equal number of specimens were cultured from each over a period of three months.

Effect of Diet on Intestinal E coli

These studies confirmed that there is a persistent *E coli* flora in healthy adults. This flora can be modified, probably through food intake. Bettelheim *et al* (1977) examined six nurses over eight weeks. During the first and last two weeks they ate a normal diet, while in the middle four weeks they ate exclusively a diet of tinned food and ultraheat-treated milk with vitamin supplements. The number of serotypes was high before the period of the sterile diet, low during it and afterwards higher than it was originally.

Animals as the Source of Human Intestinal E coli

Direct ingestion of *E coli* in food is most likely the source of human colonisation. Animal carcases are generally contaminated with their intestinal *E coli*, even under very good conditions in the slaughterhouse (Shooter *et al* 1970). There is extensive interchange of *E coli* between carcases as well. In another study (Bettelheim 1981) on a sheep slaughtering line in an abattoir in New Zealand, the animal's own rectal *E coli* tended to be washed away during the very heavy hosing down, after the removal of the intestines. However, the carcases were recontaminated by other *E coli* from the environment, which presumably were derived from the intestines of animals

slaughtered previously. The spread of both *E coli* and antibiotic resistance markers was quite extensive despite the high hygienic standard of this abattoir.

A poultry packing station was also investigated as part of the studies of Shooter *et al* (1970, 1974). *E coli* could be isolated from the chickens and their giblets throughout the processing stages. They were also isolated from the various waters used in the feather softening processes and cooling tanks. An extensive study of the contamination of pig carcases in two abattoirs (Linton *et al* 1976) found some *E coli* O serogroups throughout the stages of the slaughtering process, while others were isolated only intermittently. A generally high level of antibiotic resistance was found among the isolates. It was suggested that this reflected the state of pig rearing at that time because the pigs came from a variety of sources. The isolates did survive the chilling process and thus could be considered as potential sources of human colonisation. Although most of these studies were carried out around 20 years ago, and there have been no studies in modern abattoirs, the continuing high infection rate with Gram-negative enteric pathogens, including now *E coli* O157 and other EHEC, suggests that this contamination is still continuing.

E coli *in Food*

Cooke *et al* (1970) examined the contamination of hospital food by *E coli* and demonstrated a strong correlation between the faecal serotypes and those contaminating the food ingested by the patients. There appeared to be a continuous flux of *E coli* between the patients' food entering the ward environment and their faeces. In a survey of over 4000 samples of retail processed foods in the UK, Pinegar and Cooke (1985) noted an overall carriage rate of 12% of *E coli*. More than 25% of the cakes and confectionery and only 9% of meat and meat-based products were contaminated. These isolates from meat products were more likely to be antibiotic resistant. Over 10^3 *E coli*/g food were isolated from 27% of foods.

The Emergence of Enterohaemorrhagic *E coli* (EHEC)
Verotoxigenic E coli

Studies by Konowalchuk *et al* (1977) demonstrated that some isolates of *E coli* were able to produce a cytotoxin, which was active on Vero cells, which are derived from the kidney of a normal adult African green monkey (*Cercopithecus aethiops*). These verotoxins (VT) are distinct from both the heat labile (LT) and heat stable (ST) enterotoxins, produced by enterotoxigenic *E coli* (ETEC). They had originally been found to be produced by a number of EPEC serogroups, particularly O26 and O111, but not by all EPEC serogroups. Verotoxigenic *E coli* are known as VTEC. Since then it has been established that most EPEC do not produce VT. EPEC are characterised by the production of attachment-effacement lesions on the intestinal mucosa of susceptible animals (Moon *et al* 1983) and the EPEC attachment factor (EAF) (Knutton *et al* 1989). These characteristics are quite distinct and having been shown to be present even in archetypal strains (Robins-Browne *et al* 1993) absolutely differentiate the EPEC from the VT-producing *E coli*, although some may belong to the same O-groups. The enterohaemorrhagic *E coli* (EHEC) are those types that produce specific adherence factors in addition to the VT.

Human Disease due to EHEC

Some 40 years ago a condition known as haemolytic uraemic syndrome (HUS) was described (Von Gasser *et al* 1955). While occurring in all age groups, it has been more frequently associated with young children, in whom it is a major cause of renal failure. Many studies including those of Carter *et al* (1987) and Scotland *et al* (1988) have linked this condition with VT-producing *E coli*. Prodromal bloody diarrhoea, which occurs mainly in children, has been reported both as outbreaks of haemorrhagic colitis (Riley *et al* 1983) and as sporadic cases (Pai *et al* 1984). It has been associated

with strains of *E coli* of serotype O157.H7 which produce VT but do not produce LT or ST. Although EHEC of serotype O157.H7 have been most frequently associated with outbreaks, other serotypes of *E coli* are also found.

The Early Outbreaks due to E coli O157

It was the outbreaks in the USA in 1982 (Riley *et al* 1983) that first alerted the microbiological world to this serotype O157.H7. In the original study it was described as a "rare" serotype. As a result of this and other studies Ørskov *et al* (1987) re-examined isolates of *E coli* belonging to the O157 group that had been submitted to the International Escherichia and Klebsiella Centre since the establishment of this O-group. Only three isolates were found that also had the H antigen H7. They had been part of a batch of 39 isolates from calves with colibacillosis in Argentina. These three isolates were from the faeces of one of three animals from one farm and were the only verotoxigenic isolates among the 39. They were also biochemically similar to the O157.H7 strains, which had been isolated from a number of the outbreaks. The authors suggest that cattle may be the reservoir of these organisms. In a study on the development of diarrhoea among special-fed calves* in USA, VTEC played only a small role as causes of diarrhoea, although they were isolated from a few cases (McDonough *et al* 1994). Cryptosporidia, coronavirus and rotavirus were much more important.

The Shiga-like Toxins

Infection with *Shigella dysenteriae* type 1 can also lead to HUS. Neutralisation studies have shown that one of the serological types of VT is antigenically related to Shiga toxin (O'Brien *et al* 1982; Scotland *et al* 1985). For this reason some authors call this group Shiga-like toxins (SLT). The VT, which is neutralised by antibody to Shiga toxin, has been termed SLT I (or VT1) and the antigenically distinct cytotoxin, which has the same biological effect on Vero cells, was called SLT II (VT2). Both terms have equal validity. Some isolates produce only one cytotoxin, while others produce both (Scotland *et al* 1985). It has been demonstrated further that the production of these SLTs is mediated by phage. Specific DNA probes have been used in DNA hybridisation studies to identify the toxin-specific DNA sequences. Under stringent conditions (Willshaw *et al* 1985), no cross-hybridisation could be detected between SLT I and SLT II. The early studies on these toxins have been reviewed (O'Brien and Holmes 1987).

Biochemical Features of EHEC

March and Ratnam (1986) demonstrated that strains of EHEC belonging to serotype O157.H7 generally did not ferment sorbitol. They developed a MacConkey medium, in which sorbitol replaces lactose. Use of the sorbitol MacConkey (SMAC) agar has led to a great advance in the ability of laboratories to culture for these organisms and easily identify EHEC of serogroup O157. It has unfortunately led to the misconception that only non-sorbitol fermenting *E coli* O157 are EHEC and many other EHEC Strains have been ignored. Many had forgotten that some other *E coli* also do not ferment sorbitol. As long ago as 1952 a SMAC agar was described for the isolation of EPEC of O groups O111 and O26 (Rappaport and Henig 1952), which were rare in not fermenting sorbitol. The EHEC belonging to these O groups now being isolated do ferment sorbitol and the SMAC agar will not identify them. In addition not all sorbitol non-fermenting strains of *E coli* O157 are EHEC (Pearce *et al* 1994).

Organisms other than *E coli* can carry antigens similar to O157 including a recent Australian isolate of *Citrobacter freundii*

(Bettelheim *et al* 1993). Antigenic similarities have also been noted between the *E coli* O157 antigen and antigens from *Escherichia hermannii* and *Brucella* strains (Perry and Bundle 1990).

The problem has been made even more complex with the apparent emergence of a sorbitol-fermenting clone of EHEC O157, which has otherwise the same phenotypic and genotypic features as the non-sorbitol fermenting isolates (Karch *et al* 1993). Twelve such isolates had been recovered from children with HUS from different parts of Germany. There appear to be no easy ways at present to identify rapidly all EHEC by a microbiology laboratory.

The close relationship between the production of enterohaemolysin and the Shiga-toxins noted by Beutin *et al* (1989) could enable laboratories to screen for most EHEC, not just those belonging to serogroup O157. Bettelheim (1995) has shown that this technique can be used to select for most EHEC.

Australian EHEC

The first recorded isolation in Australia of an isolate of *E coli* belonging to serogroup O157 was made by Berry *et al* in 1983. At that time tests for the presence of VT were not made, but it is noteworthy that the O157.H- strain was an LT producer on the basis of an immunological assay. A collection of isolates recovered from patients at the Royal Children's Hospital, Melbourne, which had been tested by DNA probes for the presence of sequences determining SLT I and/or SLT II, were submitted to Fairfield for serotyping. These isolates included the first demonstration in Australia of the EHEC serotype O157.H7. It produced SLT II. There were also five isolates of the non-motile variant O157.H-, one of which produced only SLT I and four of which produced both toxins.

Other serotypes were identified including members of O groups O26 and O91, which had been found in earlier studies in other parts of the world. SLT-producing isolates of O26 had also been isolated from children with diarrhoea in New Zealand (Wilson and Bettelheim 1980).

Extensive EHEC Outbreaks

Through the period of the 1980s there were many reported outbreaks and sporadic cases of haemolytic colitis (HC) and HUS. While many were from North America, there were reports from Europe and elsewhere. Isolates of O157.H7 and O157.H-were featured most prominently, but other verotoxigenic serotypes, including particularly isolates belonging to O groups O26 and O111, were also being noted. Consumption of undercooked meat or meat products were most frequently incriminated. Many other food items can be listed as associated with cases of HUS and/or HC, including yoghurt (Morgan *et al* 1993), fresh-pressed apple cider (Besser *et al* 1993) and water (Swerdlow *et al* 1992). Even bathing in a paddling pool with an affected child (Brewster *et al* 1994) was reported as associated with an O157 outbreak. Vegetables from a manured garden have been associated with an outbreak (Cieslak *et al* 1993).

The Largest EHEC Outbreak

The major event that brought the situation into public focus was the multistate outbreak in the USA in 1992-1993 (Anon 1993; O'Brien *et al* 1993). According to these reports, 583 persons were affected across four States, with 477 cases in Washington State. Most patients had eaten at a restaurant, which is part of a chain of hamburger restaurants, or had close contact with a confirmed case. In all, 171 patients required hospitalisation, 41 developed HUS and four children died. The isolates of *E coli*, which were recovered from a number of cases, were all of serotype O157.H7. They all produced both toxins SLT I and SLT II. They were positive for the adherence factors associated with EHEC and characteristically did not ferment sorbitol.

This major outbreak highlighted that very few organisms are required to cause infection. Frozen hamburger patties from batches,

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*The calves were bucket fed a liquid milk replacer diet twice daily. Roughage was not included in the diet. Water was offered once daily.

which had been incriminated epidemiologically with the outbreaks, were shown to have fewer than 100 *E coli* O157 per patty. A retrospective study was undertaken on 37 of the affected children in Washington State (Brandt *et al* 1994) who had been admitted to the Children's Hospital and Medical Center, Seattle, during the period of the outbreak. Thirty-two children (86%) had *E coli* O157.H7 confirmed by faecal cuture and 33 (89%) had eaten at one of the restaurants of the implicated hamburger chain. Of the remaining four children, who had no contact with the chain, two had known contact with an infected patient. The age range of the patients was 1 to 15 years with a median age of 5 years. Gastrointestinal involvement, especially haemorrhagic colitis, was the main primary symptom. Both renal and haematological symptoms were evident on first presentation. Over half the children showed extrarenal abnormalities. Five patients required partial or complete colectomy and two of these subsequently died. A third child died on the way to the hospital after a seizure and cardiorespiratory arrest. Pancreatitis developed in eight patients with glucose intolerance developing in three of them. Twenty-six patients had oliguria/anuria and 21 of them required dialysis. A significant number of children developed cardiovascular complications (13), pulmonary complications (9) and/or neurological complications (6). Red blood cell transfusion was required by 28 and platelet transfusion by 20 children. The authors concluded their study by emphasising the effects that contaminated food can have on society and the need for appropriate public health measures.

An extensive microbiological study was conducted on isolates of *E coli* O157.H7 from the multistate outbreak as well as isolates from sporadic cases in Washington State occurring at the same time as the outbreak, and before and after the outbreak. Also examined were isolates from sporadic cases throughout USA before the outbreak, hamburger meat both related to the outbreak as well as unrelated to it and from cattle not associated with the outbreak (Barrett *et al* 1994). All these 233 isolates were examined by pulsed-field gel electrophoresis (PFGE) (Böhm and Karch 1992) and phage typed by the extended scheme of Khakhria *et al* (1992). All the 26 isolates from patients associated with the multistate outbreak and all 27 isolates from incriminated lots of hamburger meat had the same electrophoretic pattern and belonged to the same phage type 14. Of the sporadic isolates obtained during the outbreak, a variety of phage and electrophoretic types were found. The only isolate in this group of phage type 14 differed in its electrophoretic pattern from the outbreak isolates. After the outbreak, however, two of nine patients yielded isolates of the same electrophoretic pattern as those incriminated in the outbreak strains and of phage type 14. From the other groups of isolates there were 35 with phage type 14, which represented the most common phage type found. Only one isolate also had the same electrophoretic pattern as those incriminated in the outbreak, but this was of a different phage type. This study demonstrates that even among isolates of *E coli* O157.H7 there is a great diversity. While this permits epidemiological tracing of outbreaks, the question of possible differences in pathogenicity between differing types arises.

Details of a Small Outbreak

A small outbreak in the UK (Willshaw *et al* 1994) confirmed that the dose needed to establish infection is low. The eight patients comprised three children under 13 years, one of whom developed HUS, and five adults aged over 59 years. All patients yielded strains of *E coli* O157.H7, which produced SLT II only. All but one belonged to phage type 49 and carried a plasmid of size 56 MDa. The remaining strain was also O157.H7 producing SLT II, it carried two plasmids of sizes 56 and 36 MDa and belonged to phage type 2. A remaining raw beefburger, which was the only food item that could be epidemiologically linked to the patients, also yielded *E coli* O157.H7 producing SLT II, of phage type 49 and carrying a plasmid of size 56 MDa. The total viable bacterial count of this beefburger was 5.3×10^4/g, the coliform count < 40/g, the *E coli* count < 20/g,

the *E coli* O157 count was incredibly < 2/25 g. This stresses as no other recent study the very low infective dose of these pathogens for humans.

Sporadic Cases of EHEC

Apart from the many other reported outbreaks from various parts of the world, there have been numerous sporadic cases. A recent report from Utah, USA (Siegler *et al* 1994) reviewed cases of HUS from 1971 to 1990. It is particularly relevant to be able to survey these occurrences in a specific area over the critical period in which the strains of *E coli* O157.H7 are believed to have first emerged. All children with HUS under 18 years, who had resided in Utah for at least two weeks before onset were identified by a computerised registry of HUS cases between 1971 and 1990.

During the study period 157 cases of HUS were identified. This represents an incidence of 1.42 per 100 000 population. There appears to have been an increase during the 1970s from less than ten *per annum* to about ten *per annum*. The prevalence diminished again in the early 1980s to below five *per annum*. It increased to a peak of 20 in 1987 and then again stayed around ten till 1990. The 1987 peak was associated with two clusters of cases linked to an *E coli* O157.H7 outbreak due to a common source. Most cases occurred in summer. The highest prevalence of HUS was among children aged two years or less. No significant difference between incidence in urban and rural cases could be ascertained, nor between family size, associations with agriculture or presence of dairy cattle. A weak association was noted with increased incidence and per capita income.

In about 90% of the children a diarrhoeal prodrome was noted and in 74% was there bloody diarrhoea. Seizures occurred in 9% before the diagnosis of HUS. From 1987 onwards stool culture results for *E coli* O157.H7 were available for 29/50 cases and 18 (62%) yielded this pathogen. There was a significant association of high severity of disease with infancy (< 2 years), with prodromal anuria and with a white blood cell count > 20 000 $\times 10^9$/L. The authors defined a bad outcome if the following applied: death, end-stage renal disease (ESRD) and brain damage. This was noted in 11% of the cases and it included six (4.3%) deaths during the acute phase and one late death. Severity of acute illness was predicitive of a bad outcome. Chronic abnormalities were found in about half the 72 cases for whom follow-up information was available.

Other Serotypes of EHEC

Other EHEC Serotypes in Australasia

While strains belonging to the *E coli* O157 serogroup have been predominantly associated with EHEC, there are many other serotypes that can also carry these virulence factors. This is particularly so in Australia and New Zealand. As long ago as 1980 EHEC of serotype O26.H11, O26.H21 and O39.H8 were associated with cases of diarrhoea in New Zealand (Wilson and Bettelheim 1980). Throughout the 1980s there were occasional reports of EHEC isolations in Australia and it appeared that members of the O111 serogroup were particularly prominent (Gunzburg *et al* 1988; Pryor *et al* 1990). The latter study also described the isolation of a strain O157.H7 from a case of haemorrhagic colitis in a 59-year-old man from Sydney.

E coli O111 Outbreaks in Italy

The O111 serogroup has been largely neglected. The first report of an outbreak was published in 1994 (Caprioli *et al* 1994). It describes a small outbreak in Italy in 1992, the first reported Italian EHEC outbreak. It notes a number of sporadic cases caused by *E coli* O111.H- as far back as in 1988. It appears that cases caused by O111 have also occurred throughout many parts of Europe, particularly Italy, France and Germany (A Caprioli, personal communication).

More recently a community-wide outbreak of HUS was reported from a large area of Northern Italy over the period from March to

October 1993 (Tozzi *et al* 1994). Mainly serological evidence suggested that most cases were caused by *E coli* O111, some by *E coli* O157 and one child yielded a strain of *E coli* O86.H40 that produced SLT II. Exposure to live animals, particularly chickens were considered an important risk factor. Exposure to cattle was less of a risk factor.

Other EHEC Serotypes round the World

The three serogroups O26, O111 and O157 have appeared as the most common EHEC in a recent Chilean study (Cordovéz *et al* 1992). This detailed controlled study revealed eight cases of HUS. Three of these cases yielded toxigenic isolates of O157, three yielded toxigenic O111 isolates and two toxigenic O26 isolates. Two control children were carrying toxigenic *E coli* of serogroup O26.

A careful examination of publications over the last few years reveals that there are many EHEC serotypes apart from those belonging to O groups O26, O111 and O157. While many have been isolated from healthy animals and not associated with disease in humans or animals (Beutin *et al* 1993), there have been reports of a number of diverse EHEC serotypes associated with human disease. Recently in Seattle, USA, Bokete *et al* (1993) investigated children with gastrointestinal symptoms typically associated with EHEC. The following serotypes were isolated: O153.H2; O68.H-; O26.H- and O-.H11. A multicentre study from France found from a number of children with HUS strains of *E coli* O103.H2 producing SLT I (Mariani-Kurdjian *et al* 1993). In Australia a great variety of serotypes have been associated with cases of HUS, including O26.H11; O48.H21; O91.H10; O98.H-; O111.H2; O111.H8; O111.H-; O112a,b.H2 and O165.H- (Goldwater and Bettelheim 1995). This survey noted that isolates belonging to O-group O111 were particularly prominent. It was therefore not surprising that the first Australian outbreak of HUS should be associated with strains of O111 (Cameron *et al* 1995).

EHEC in Animals and the Food Chain

Bovine Carriage of EHEC

It appears that EHEC strains belonging to serogroup O157 are associated with cattle and possibly other domestic meat animals. Chapman *et al* (1993) isolated *E coli* O157 from 84 (4%) of 2103 bovine rectal swabs in an abattoir in England. The positive cattle, which were apparently healthy, were from many parts of England. *E coli* O157 isolates were cultured from 30% of carcases of these rectal swab positive animals. In addition the carcases from 8% of rectal swab negative animals yielded *E coli* O157 strains. The potential hazard of such carcases for human consumption was stressed. *E coli* O157 have been isolated from cattle in Canada (Borczyk *et al* 1987) and USA (Martin *et al* 1986; Wells *et al* 1991).

However, while serotype O157.H7 has been recovered from cattle it may not necessarily occur frequently. A study of 1131 dairy cows and 659 calves in Ontario, Canada (Wilson *et al* 1992) specifically looked for SLT-producing *E coli*. The infection rate in cows was as high as 60% and in calves as high as 100% in some farms. The total prevalence for cows and calves was 9.5% and 24.7%, respectively. None of the 206 VTEC belonged to serotype O157.H7. In the State of Washington, USA, an extensive survey of faecal carriage of *E coli* O157.H7 in 3570 dairy cattle from 60 herds found that ten cows carried these organisms. These cows came from five of the herds representing a herd carriage rate of around 8% (Hancock *et al* 1994). Similarly ten of 1412 pastured beef cattle were found to carry these strains. This represented four of 25 herds (16%).

A study by Dorn *et al* (1993) on isolates of VTEC isolated cattle including cases of diarrhoea in calves between 1983 and 1989 in USA, failed to find any isolates of serogroup O157. Most of the VTEC isolated belonged to serogroup O111. Others found included representatives of serogroups O5, O26, O45, O69 and O103. Of the 36 calves that yielded these VTEC, 12 had bloody diarrhoea and seven of them carried O111.H-. All the isolates readily fermented

sorbitol and most produced only SLT I. A number of the serotypes found in this study have been reported in earlier studies associated with human disease including O5.H-, O26.H11, O45.H2 and O111.H-. The role of these VTEC as well as their relation with strains of O157 within the bovine intestinal tract of cattle still has to resolved. Frank *et al* (1994) report the consistant colonisation of tonsils of healthy cattle by *E coli*. The only two SLT-producing strains, which were among the 124 isolates from 87 calves, produced SLT I and did not belong to serogroup O157. They were not further characterised. It is suggested that the tonsils may be an important source of VTEC for human infection.

The possibility that certain cattle management techniques may contribute to the maintenance of these organisms in animals was indicated and further studies are necessary. A recent development of a sandwich ELISA, based on the use of monoclonal antibodies (Ball *et al* 1994), may provide the means of directly testing for the presence of VTEC in cattle and other animal faeces. This study, which was performed in Northern Ireland, revealed that of the 304 samples from enteritis cases, 74 (24%) were positive and of the 113 samples from healthy animals, 35 (31%) were positive for VTEC. This again emphasises the widespread nature of these *E coli* in the domestic animal population. It also confirms the lack of correlation of VTEC carriage with enteritis in cattle.

A number of similar studies have raised the question about the characteristics that cause some *E coli* to be human pathogens. It appears that toxigenic strains of *E coli* O157 as well as many other serotypes occur in domestic animals, particularly cattle, but only a very limited number of serotypes is associated with human infection. A recent outbreak of diarrhoea due to strains of O157.H7 among Inuit people in the Northwest Territories of Canada (Orr *et al* 1994) might have been associated with eating caribou meat, suggesting the possibility that these animals are also carriers of these *E coli*.

In attempting to answer why some but not all VTEC cause human disease, Barrett *et al* (1992) examined a selection of strains for various virulence factors, but were unable to determine what really constitutes an EHEC that will cause human infection. This issue was further addressed by Beutin *et al* (1995), who showed that the SLT-producing *E coli*, isolated from healthy domestic animals are a very heterogeneous group. Some species specificity was noted but the factor(s) which may be involved in human pathogenicity were not elucidated.

The Rumen as a Habitat for EHEC

It is perhaps noteworthy that *E coli* O157.H7 strains grow better in the rumens of fasted cattle than in well-fed cattle (Rasmussen *et al* 1993). This suggests that treatment of animals or restriction of food intake before slaughter may affect carriage of these organisms. It has been demonstrated that under the conditions in the rumen *E coli* can transfer plasmids to each other (Scott and Flint 1995). While that study concentrated on plasmids carrying antibiotic resistance, it is conceivable that plasmids carrying virulence factors are also transmitted.

Relationship of Human to Bovine Types

There have been differing reports on the distribution subtypes of *E coli* O157 from human patients and cattle. While Paros *et al* (1993) in USA found that 90% of human isolates did not match those from cattle, Chapman and Siddons (1994) in the UK found a strong correlation between human and bovine isolates. These differences are likely to be associated with variability in sampling.

Chickens as Carriers of EHEC

Chickens are able to be colonised by these *E coli* and poultry can also be a source for human infection (Stavric *et al* 1993). When one-day-old chicks were infected by mouth with a strain of *E coli* O157.H7, the gut became colonised for 10 to 11 months (Schoeni

and Doyle 1994). The strains were recovered from the shells of eggs laid by faecal shedders of these bacteria. The yolks or whites of these eggs were not infected.

Survival of EHEC in Food and on Meat

The survival of EHEC and their transmission through the food chain has been investigated. EHEC appear to be more acid resistant than other *E coli*, explaining their survival in some acid-preserved foods. It may also indicate a greater ability to survive stomach acidity, thus explaining the low infective dose. Abdoul-Raouf *et al* (1993) examined the growth of *E coli* O157 in beef and beef products in the presence of various organic acids at different temperatures. While the acidity of most mayonnaise formulations retarded the growth of many microorganisms, it could not be relied to control the growth of *E coli* O157 strains.

The inhibitory effects of other organic acids were not as great on *E coli* O157 as they were on other organisms. Acetic acid has been suggested for use as a sanitiser spray on beef carcases because it reduces the numbers of *E coli* O157 (Dickson 1991) on the surface.

The widespread occurrence of VTEC in foods was demonstrated by Samadpour *et al* (1994). They examined retail fresh seafood, beef, lamb, pork, and poultry from grocery stores in Seattle, WA, USA. Using DNA probes for SLT I and SLT II sequences, a high positivity rate of the various samples was noted, with five of eight veal samples (63%) being the highest rate. Other rates were 23% for beef, 18% for pork, 48% for lamb, 12% for chicken, 7% for turkey, 10% for fish and 5% for shellfish. None of the isolates belonged to the O157 serogroup but there were strains belonging to serogroups that had been associated with human disease including O6, O91, O113 and O163. The strains belonging to these serogroups were isolated from beef (O6, O113 and O163) or pork (O91). It should be noted that VTEC including strains belonging to serogroup O157 have been isolated from retail meats as long ago as 1987 (Doyle and Schoeni 1987).

Irradiation may provide the only way by which these bacteria can be excluded from the food chain. Studies have shown that the application of 2.5 kGy of gamma irradiation will effectively eliminate them (Clavero *et al* 1994).

Conclusions

E coli *in Humans and Animals*

E coli colonise the intestines of humans and many animals shortly after birth. There is a great variety of types, which have been characterised by serotyping, biotyping and other techniques.

Most studies on the ecology of these organisms have demonstrated a constant flux of *E coli* in these environments. New types impinge and may colonise replacing existing types. Generally the source of new types is food. Usually a large inoculating dose is required to establish colonisation with commensal *E coli*. While there is some overlap, it seems probable that there are species-specific types of *E coli*. There is a strong species specificity among the pathogenic groups of *E coli*.

Emergence of EHEC

Recently the EHEC have emerged. Serotype O157.H7 and its non-motile variant have been given most prominence. Strains belonging to these two serotypes O157.H7 and O157.H-, which have been isolated from many parts of the world, have very similar biochemical characteristics and may be belong to one clone. This clone has been associated with a number of outbreaks, but the importance of other serogroups, most notably O26, O111 and O113, should not be overlooked. The O157 clone, which was almost unknown before 1980, appears to have colonised many domestic animals throughout the world. It is able to infect humans with an infective dose well below 100 bacteria. This suggests that it must

have some unique colonising ability absent in many other *E coli*. It is possible that this is the characteristic noted among the EPEC clone in the small outbreak described in 1983 (Bettelheim *et al* 1983b).

Cattle as Main Source of EHEC

The studies that have been done on cattle and other animals have revealed that many VTEC are present in their faeces. Strains of O157 have been also been found, albeit in small numbers. Human disease appears to have been caused by a very limited number of toxigenic *E coli* serotypes. For none of these human pathogenic types is there much evidence of association with disease in animals. The outbreaks associated with serotypes other than O157, such as the ones described from Italy involving O111 strains, have not been linked directly to animals. Studies on the recent VTEC O111 outbreak in Adelaide, South Australia, due to VTEC O111, while strongly linked to eating a type of sausage, have not yet led directly to the source of the bacteria.

The Human Factor

This review has concentrated on the bacteria. However, it is important to consider the emergence of these bacteria in the context of the changes in human behaviour over the last few decades. There have been many aspects of lifestyle that would influence the emergence of such pathogens. These could include the increasing urbanisation and increasing movement of people and goods, particularly foods around the world. A simple path for the spread of a pathogen like *E coli* O157 to a new environment could be that an individual carrying it jets to the new area, his/her faeces enter the sewage, which is inadequately treated before discharge into the sea. The *E coli* infect the sea birds. When the weather at sea is rough, they feed and defecate on pastures where cattle and sheep graze. The route is then short from the pasturing animals to human infection.

Levine and Levine (1994) discussed these human factors in a recent review. The extensive commercialisation of food production and service, as well as the changing role of women in domestic food production, are seen as major factors. The multistate outbreak in western USA occurred only because the meat patties were prepared in bulk in one plant and shipped to many restaurants. The meat for these patties came from a variety of abattoirs, which drew their supplies from many farms. The extensive development of fast-foods and the sale and storage of frozen foods, the use of microwave ovens to heat foods rapidly may all contribute to the provision of new niches for potential pathogens to enter or survive in the food chain.

While some of the changes in lifestyle will reduce the importance of the traditional diarrhoeal diseases, they may well create niches for the emergence of new ones. EHEC appear to have developed to fill some of these niches. By being in most ways no different, from any *E coli*, standard techniques of food examination will fail to detect them. Similarly, veterinarians will not detect them because infected animals are rarely ill. Only a small inoculum dose is required to infect humans with EHEC. This makes the detection of infected foods very difficult. Unless major changes in the whole food chain are instituted it is likely that the problem of EHEC may get worse before one can see any improvements.

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