**Escherichia coli O157:H7. How one organism has changed attitudes to food safety and focussed attention on the ruminant host**

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**Summary**

*Escherichia coli* O157:H7 has emerged as a major human food–borne pathogen over the last two decades. Unlike many food–borne pathogens, this organism can cause life–threatening disease and is a particular threat to children through acute and chronic kidney damage. As a consequence of the increasing consumer concern over food safety, Governments have acted to tighten food safety regulations and to investigate on–farm sources of this organism. Cattle have been identified as the major source, either through the faecal contamination of beef carcasses or through manure contaminating crops and drinking water.

**Keywords:** *Escherichia coli*, food safety, ruminant, probiotics

**Introduction**

*Escherichia coli* O157:H7 was first recognised as a foodborne pathogen in 1982 from an outbreak of haemorrhagic colitis (HC) in the USA (Riley et al. 1983) and has since been associated with other outbreaks and sporadic cases of illness worldwide. This is a recently evolved human pathogen and illnesses caused by this pathogen have been recorded in most countries, although not commonly in Australia. There is evidence from a retrospective review by the US Center for Disease Control, of over 3000 *E. coli* isolates collected between 1973–1983, that this organism had previously been associated with rare sporadic infections (USDA 1997). In the USA, *E. coli* O157:H7 has been estimated to cause 73,480 human cases of illness each year (Mead et al. 1997). The disease in humans

*E. coli* O157:H7 is associated with the development of the serious diseases HC and Haemolytic Uraemic Syndrome (HUS). HUS occurs in about 10% of *E. coli* O157:H7 infections and has a mortality rate of 2–10% (Law 2000). In Scotland in 1996, there were 21 deaths in over 400, mainly elderly, people infected (Ahmed and Donaghy 1998). The potentially high mortality associated with O157:H7 distinguishes this organism from other strains of *E. coli*. Those affected initially have watery diarrhoea but this can progress to haemorrhagic diarrhoea and abdominal pain. A number of patients, particularly children and the elderly, develop HUS characterised by kidney failure, microangiopathic haemolytic anaemia and thrombocytopenia. HUS is a major cause of renal failure in children. Some people exhibit neurological symptoms and can suffer strokes.

Orally ingested Shiga–toxin producing *E. coli* (STEC) pass through the acid environment of the stomach and colonise the intestine by adhering to the intestinal wall principally in the colon and distal small intestine. The organisms remain in the gut but release Shiga toxins which pass through the intestinal epithelium into the blood stream (Paton and Paton 1998). Monnens (2000) demonstrated that the Shiga toxin binds to human polymorphonuclear leucocytes and that the toxin can then transfer to microvascular endothelial cells causing inhibition of protein synthesis and cell death. Vascular endothelial cells of the kidney glomerulus and brain are considered major targets. Interestingly, Shiga toxins do not bind to bovine leucocytes (Monnens pers. comm.) and systemic disease does not occur in cattle.

*E. coli* O157:H7 has been primarily associated with ground beef. Faecal contamination of carcasses during animal production and slaughter is considered the main source of organisms in meat (Elder et al. 2000). *E. coli* O157:H7 has also been associated with sprouts, lettuce, unpasteurised apple juice, unpasteurised milk, yoghurt, cheese, and drinking water usually as a result of contamination with cattle manure. Other sources have been direct contact with infected humans, or via swimming water (USDA 1997) and contact with animals.
during farm visits (Mainil 1999). A large outbreak occurred in Walkerton, Canada in 2000, through the contamination of municipal drinking water by cattle manure. Approximately 30% of the town’s population of 5000 were affected and nine people died (McQuigge et al. 2000). Cattle are considered the main source of \textit{E. coli} O157:H7, but the organism does not cause disease in cattle.

**The Shiga–toxin producing \textit{E. coli}**

O157:H7 is the most notable \textit{E. coli} in a group known as the STEC. Enterohaemorrhagic \textit{E. coli} (EHEC) are STEC that have been isolated from human cases of enterohaemorrhagic disease. There is a wide range of virulence among STEC and it appears that not all strains found in animal reservoirs are pathogenic for humans. Many of the virulence factors are encoded on mobile genetic elements such as phages, plasmids and pathogenicity islands and are likely to be transferred between bacteria. The Shiga toxins are thought to be responsible for the clinical manifestations of HC and HUS. The genes encoding Shiga toxins are encoded on bacteriophages. In addition to Shiga toxins, EHEC commonly possess the intimin (\textit{eaeA}) gene which facilitates attachment to cells and an EHEC–specific plasmid encoded haemolysin (\textit{hlyA}) gene. It is generally considered that \textit{E. coli} O157:H7 is more virulent than other STEC, although the reason is not yet known. Shiga toxin 2 and the adhesin intimin appear to be the most important virulence factors for \textit{E. coli} O157:H7 but there may also be factors yet unidentified. The infective dose in humans is believed to be as low as 50–100 bacteria, or even lower (Armstrong et al. 1996).

\textit{E. coli} O157 may be more robust and able to survive in the environment than other STEC. Barker et al. (1999) demonstrated that this organism can survive and replicate in a common environmental protozoan. The acid tolerance of this organism, which will survive at pH 2.5, gives it survival advantages in the gastrointestinal tract as well as in food products such as apple cider, yoghurt, mayonnaise and fermented meats such as salami. This ability to survive an acidic environment is not unique to \textit{E. coli} O157; other STEC also have this characteristic (Waterman and Small 1996).

It is estimated that approximately 25% of HUS cases in the USA are caused by non–O157 STEC (Johnson et al. 1996); these are often overlooked because of testing methods used. Numerous studies have shown that the isolation rates of non–O157 STEC are higher than O157 STEC in both cattle faeces and beef (Johnson et al. 1996; Willshaw et al. 1993). It can be assumed from these findings that humans are exposed to non–O157 more frequently than they are to O157 STEC. The incidence of HUS in Australia is lower than in North America and is more commonly associated with \textit{E. coli} O111 than with \textit{E. coli} O157 (Henning et al. 1998). There are few cases of disease in humans in Australia associated with O157:H7; other serotypes such as O157:H–, O111:H–, O113:H21 and O26:H11 predominate (Goldwater and Bettelheim 1994; Robins–Browne et al. 1998).

**The origins of \textit{E. coli} O157:H7**

\textit{E. coli} O157:H7 has emerged as a human pathogen over the last two decades. Studies of this organism indicate that approximately 4.5 million years ago the precursor of today’s O157:H7 and O55:H7 descended from a common ancestor. Subsequently O157:H7 lost its ability to ferment sorbitol, acquired the Shiga toxins, Stx2 then Stx1, and possibly other virulence factors (Reid et al. 2000). It is open to speculation which event in the evolution of this organism caused it to explode as a major human pathogen. In the USA two distinct lineages, present in both humans and bovines, have been identified by octamer–based genome scanning of isolates (Armstrong et al. 1996; Kim et al. 1999). Kim et al. (1999) suggest that one of these lineages may be less virulent for humans or less efficiently transmitted to humans from bovines. Perna et al. (2001) have fully sequenced the genome of the strain associated with human disease from contaminated hamburgers in the USA in 1982. This genome was compared with that of the non–pathogenic laboratory strain \textit{E. coli} K–12. A total of 1387 new genes were identified in O157:H7 including candidate virulence factors, alternative metabolic factors, several prophages and other new functions. These findings demonstrate that lateral gene transfer is extensive and that enterobacterial genomes are particularly subject to recombinational evolution (Perna et al. 2001).

The prevalence of STEC is apparently greater in ruminants than in other types of domestic animals (Beutin et al. 1993). The majority of STEC are probably normal intestinal flora in ruminants. In an Australian study of slaughter–age animals from 215 herds and flocks, STEC were cultured from 58% of mutton sheep properties, 46% of prime lamb properties, 36% of pasture beef properties, 14% of feedlot properties and 10% of dairy cattle properties. \textit{E. coli} O157:H7 was only isolated from one animal at one feedlot (Vanselow and Hornitzky 2001). In other Australian studies using specific detection techniques, O157:H7 has been found more commonly. Cobbold and Desmarchelier (2000) isolated STEC from 16.7% of dairy cattle faecal samples and of these 11.2% were \textit{E. coli} O157:H7 (i.e. a prevalence of 1.5%). In US studies, individual animal prevalence estimates are 0–3%. Similar individual animal estimates have been made for other countries, for example England 1% and Germany 0.9% (USDA 1997). In longitudinal studies, herd prevalence in the US is estimated to range from 22% to 100% (USDA 1997). Reported levels of \textit{E. coli} O157:H7 in cattle in the US are apparently increasing, but this may reflect the more sensitive detection techniques being used. Many STEC appear to have a preference for particular animal species, e.g. cattle or sheep (Djordjevic et al.
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2001; Hornitzky et al. 2001), with multiple serotypes occurring together and probably in balance in the gut (Vanselow and Hornitzky 2001). Under environmental conditions such as dietary changes that can alter gut pH and concentrations of volatile fatty acids, one serotype may be advantaged over others. It has been speculated that the use of ionophors in cattle feed may increase the prevalence of O157:H7 or was involved in its evolution, but this has not been proven (USDA 1997; Dargatz et al. 1997). E. coli O157:H7 is not species-specific; it has been isolated from both cattle and sheep and to a lesser extent from pigs and other domestic and wild species (Wells et al. 1991; Kudva et al. 1996; Cornick et al. 2000; Sargeant et al. 1999). Gut colonization in cattle is transient with a median shedding duration of less than 30 days (Besser et al. 1997).

Retrospective studies have reported the existence of STEC in collections of bovine E. coli from the 1960s (Mainil 1999) but E. coli O157:H7 appears to have recently found a niche in the gastrointestinal tract of cattle, particularly in the intensive cattle industry of North America. It may have spread from contact with another ruminant species such as deer, or inadvertently been favoured in animals fed high carbohydrate diets and been able to spread easily in large densely managed cattle populations. Cornick et al. (2000) postulate that STEC serotypes persist in some animals and that these animals become reservoirs. A few persistent shedders in a herd would be enough to transmit STEC to naïve animals. Some STEC serotypes can cause diarrhoea in calves but the majority of STEC, including E. coli O157, do not cause disease in cattle.

How O157:H7 has affected attitudes and policies to food safety

The largest outbreak in the USA occurred in 1993 in four States. There were 732 human cases and 4 deaths in young children who had eaten hamburgers in fast food restaurants. These illnesses and deaths marked the change in US policy towards food safety. In 1994, the Food Safety and Inspection Service of the USDA declared that raw ground beef contaminated with E. coli O157:H7 is adulterated and must be further processed to kill the pathogen or be destroyed. Previously, bacteria had not been considered an adulterant. This change in policy resulted in some massive recalls of ground beef. In 1997, Hudson’s Meats USA recalled 25 million pounds of hamburger mince following human infections. In 1997, President Clinton announced the President’s National Food Safety Initiative for more surveillance, research and control procedures. Regulations regarding food preparation were tightened, and Hazard Analysis Critical Control Point (HACCP) systems were refined to deal with this new pathogen. For example the cooking of meat patties is now controlled so that the centre of the patty reaches a temperature to kill bacteria. Pasteurisation is now routine for juices and cider. Irradiation is increasingly being adopted as a control measure to eliminate bacteria and viruses from many food products. Funding for research into food safety has been increased. This research is investigating all levels of food production from ‘paddock to plate’ especially in view of the outbreaks of E. coli O157 associated with environmental contamination. Countries such as Australia that export meat to the USA are also affected by these changes in policy. The US Center for Disease Control (CDC) in collaboration with US State health departments and Federal food agencies is enhancing national surveillance for foodborne diseases.

In 1997 a national network commenced ‘PulseNet’, using pulsed-field gel electrophoresis to identify subtype strains of E. coli O157:H7 (Tauxe 1997). In addition, population based active surveillance for HUS began in FoodNet sites in 1997 (Griffin 2000). Similar networks are now established in Europe. In 1997 the EU–wide network for surveillance of human salmonella infections was extended (Smith 2000) to cover STEC.

Canada has also experienced outbreaks of E. coli O157:H7 in humans related to meat and other foods. The outbreak that was associated with cattle manure contaminating the municipal drinking water supply has further identified cattle manure as an environmental contaminant. A similar episode occurred in New York State through manure contaminating drinking water wells. There is now considerable research into the survival of this organism in the environment and in the biofilms in water pipes (McQuigge et al. 2000).

In the UK, changes have been implemented following an outbreak in Scotland that claimed 21 lives and affected over 400 people, mainly elderly, caused by mishandling of cooked and raw meat. A committee of experts chaired by Professor Hugh Pennington was set up in November 1996 to advise the Government on the implications for food safety and the general lessons to be learned from that outbreak. The committee’s recommendations included (Pennington 1997):

- new licensing systems for butcher’s shops
- physical separation of raw and cooked product
- use of separate staff for handling cooked and uncooked product where possible
- training in food hygiene
- HACCP system documenting hazard analysis, record keeping, temperature control, monitoring record keeping by wholesalers to facilitate product recall
- research needed on the prevalence and incidence of E. coli O157 in livestock
- more accurate methods of typing E. coli strains using DNA fingerprinting.
In Australia in early 1995, 20 children in South Australia were diagnosed with HUS associated with STEC O111:H– in locally produced fermented sausage. Of these 20 children, one died, 18 required renal dialysis and 5 had significant kidney impairment 12 months after discharge from hospital (Henning et al. 1998). This had an immediate impact throughout Australia on the sales of packaged salami. In the first 5 months sales dropped nearly 50% but, more importantly, sales did not fully recover over following years (Adams 1998). Subsequent studies revealed there was wide variation in the quality of product available and a wide range of manufacturing methods. The smallgoods industry worked with Government and scientific organisations to draft new standards for the fermentation of salami and develop HACCP plans for this process. As a result many ‘below standard’ small producers are no longer in business.

The largest outbreak worldwide occurred in Japan in 1996. Children were predominantly affected with 6000 cases of HC and over 100 cases of HUS. The most likely source identified was radish sprouts in mass produced school lunches with cattle manure implicated as a contaminant. This outbreak emphasised that foods other than meat can harbour this organism and prompted considerable research in Japan into the disease and its control and treatment (USDA 1997; Paton and Paton 1998).

Management, feeding practices and therapeutics that affect the faecal shedding of O157:H7

Alterations in farm management or feeding regimes may reduce the risk of these bacteria entering the human food chain. Potential intervention strategies on–farm include feed and water hygiene, reduction in contact between animals especially between species, selection of genetically resistant animals, vaccines to specific serotypes, the use of probiotics to compete with STEC, genetically resistant animals, vaccines to specific serotypes, the use of probiotics to compete with STEC, specifically may be prolonged on a roughage diet. Any sudden dietary changes or period of fasting can be associated with higher excretions. Dargatz et al. (1997) suggested that digestion dynamics, including gastrointestinal transit times or fermentation patterns may be involved. The short chain fatty acids in the rumen (e.g. acetate, propionate and butyrate) are weak acids with bacteriocidal properties. Duncan et al. (1999a) showed that propionate was more inhibitory than acetate or butyrate for E. coli O157:H7. Until there is greater understanding of the ecology of these organisms in the ruminant gastrointestinal tract, dietary recommendations to reduce the excretion of pathogenic STEC cannot be made.

Probiotic bacteria have been used successfully to reduce excretion of E. coli O157:H7 in cattle. Zhao et al. (2000) demonstrated a reduction in the carriage and faecal excretion of E. coli O157:H7 in experimentally infected beef cattle by feeding five strains of competitive non–pathogenic E. coli; the competitive exclusion E. coli could reduce or eliminate faecal shedding of O157:H7 to undetectable levels in most adult grain–fed cattle within nine days of receiving the competing bacteria. Takahashi et al. (2000) demonstrated a beneficial effect from feeding probiotics containing Clostridium butyricum, Lactobacillus plantarum and Streptococcus faecium on the levels of E. coli O157:H7 in cattle faeces. Lema et al. (2001) demonstrated that the total number of O157:H7 shed in faeces of experimentally infected lambs was reduced by including lactic acid producing bacteria in their feed. Six groups of 5 lambs were fed daily for 7 weeks, diets with and without a bacterial supplement. O157:H7 was shed continuously by all groups throughout the 7 week period but there were significant differences in

been considerable debate on the effect of diet on E. coli, acid resistant E. coli and specifically E. coli O157:H7 (Dargatz 1997; Kadwa et al. 1997; Cray et al. 1998; Jordan and McEwen 1998; Diez–Gonzalez et al. 1998; Harmon et al. 1999; Hovde et al. 1999; Buchko et al. 2000). Diez–Gonzalez et al. (1998) conducted a study of 61 cattle which indicated that great supplementation could increase total and acid–resistant E. coli numbers. Hovde et al. (1999) compared hay–fed with grain–fed steers experimentally infected with E. coli O157:H7 and reported that hay–fed animals shed E. coli O157:H7 for significantly longer than did those grain–fed. Buchko et al. (2000) found no significant difference in shedding of E. coli O157:H7 in naturally infected cattle that were fed either concentrate or forage diets, or during fasting; re–feeding following a 48 h fast did significantly increase shedding. A similar finding was made by Cray et al. (1998). Calves fasted for 2 days before inoculation with E. coli O157:H7, shed significantly greater numbers of that organism than those non–fasted.

In summary, feeding roughage is apparently associated with lower E. coli levels compared to feeding a concentrate diet, but the excretion of E. coli O157:H7 specifically may be prolonged on a roughage diet. Any sudden dietary changes or period of fasting can be associated with higher excretions. Dargatz et al. (1997) suggested that digestion dynamics, including gastrointestinal transit times or fermentation patterns may be involved. The short chain fatty acids in the rumen (e.g. acetate, propionate and butyrate) are weak acids with bacteriocidal properties. Duncan et al. (1999a) showed that propionate was more inhibitory than acetate or butyrate for E. coli O157:H7. Until there is greater understanding of the ecology of these organisms in the ruminant gastrointestinal tract, dietary recommendations to reduce the excretion of pathogenic STEC cannot be made.
the numbers being shed: diets containing *S. faecium* (3.5 log_{10} Colony Forming Units [CFU] *E. coli* O157:H7 per gram of faeces) or a mixture of *S. faecium*, *Lactobacillus acidophilus*, *L. casei*, *L. fermentum* and *L. plantarum*. (2.3 log_{10} CFU per gram of faeces) produced significantly lower shedding levels (P<0.05) than other groups and the control group (5.6 log_{10} CFU per gram of faeces). Duncan *et al.* (1999b) identified 11 strains of *Psuedomonas aeruginosa* that inhibited the growth *in vitro* of *E. coli* O157.

Similarly, oral administration of bacteriophages has been shown to have beneficial effects. Waddell *et al.* (2000) demonstrated that oral administration of a mixture of six bacteriophages capable of lysing most *E. coli* O157:H7 strains significantly reduced the shedding of O157:H7 by calves. Jordi *et al.* (2000) demonstrated that specific colicins could inhibit five pathogenic STEC serotypes *in vitro* and intend testing these in cattle. Colicins are bacteriocins (toxic products of bacteria) that specifically inhibit *E. coli* and closely related bacteria.

**Conclusions**

It may be possible to reduce levels of specific STEC, but it is unlikely that all STEC could be eliminated because they are commonly found in cattle and sheep and most would be normal intestinal flora. *E. coli* O157:H7 is a recently emerged strain that can reside in the ruminant gastrointestinal tract, to identify which are pathogenic for humans, to understand how an organism such as *E. coli* O157:H7 evolves, and how to control shedding in faeces. In Australia, although O157:H7 has been identified in both cattle and sheep, the incidence of the disease in humans is low and Australia has yet to experience the consequences of a large outbreak in humans. Food handling and preparation practices may differ from those of other countries, or the prevalence of *E. coli* O157:H7 in Australian cattle and sheep may be lower, or the strains present may not be as virulent as in other countries.

**References**


