E.coli O157:H7

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Introduction

According to the Latin proverb, we are born between the urine and 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 colicommune* (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.

Most *E.coli* strains are harmless commensals, however, some strains are pathogenic and cause diarrhoeal disease. *E.coli* strains that cause diarrhoeal illness are categorized into specific groups based on virulence properties, mechanisms of pathogenicity, clinical syndromes and distinct O: H serogroups. These categories include enteropathogenic *E.coli* strains (ETEC), enteroinvasive *E.coli* strains (EIEC), diffuse adhering *E.coli* strains (DAEC), enteroaggregative *E.coli* strains (EAggEC) and enterohaemorrhagic *E.coli* strains (EHEC).

EHEC were first identified as human pathogens in 1982, when *E.coli* of serotype O 157:H7 was associated with two outbreaks of haemorrhagic colitis. All EHEC produce factors cytotoxic to African green monkey kidney (VERO) cells, which have been described as verotoxins (VTs).E.coli O157:H7 and many serotypes of *E.coli* have subsequently shown to produce VTs; hence they have named VT-producing *E.coli* (VTEC).

E.coli O157:H7

The first confirmed isolation of *E.coli* O157:H7 in the United States was in 1975 from a California woman with bloody diarrhoea.The first reported isolation of *E.coli* O157:H7 from cattle was from a less than 3 week old calf with colibacillosis in Argentina in 1977. The bacterium was first identified as a human pathogen in 1982, when it was associated with two food borne outbreaks of Hemorrhagic colitis.

Since then O157 VTEC have been identified in many outbreaks and in sporadic cases of bloody diarrhoea in North America and Great Britain and a close association has been established between VTEC and haemorrhagic uremic syndrome (HUS).

Characteristics of E.coli O157:H7

Most strains of E.coli O157:H7 possess several characteristics uncommon to most other E.coli.

(i) Acid tolerance: Unlike most food borne pathogens, *E.coli* O157:H7 uniquely tolerant to acidic environments. Acid tolerance is a complex phenomenon, both growth phase dependant and inducible.*E.coli* cells in stationary phase of growth are substantially more acid tolerant than cells in the exponential phase. This increased tolerance is associated with expression of genes regulated by the rpoS sigma factor operon(Cheville *et al.*1996;Rowbury,1998; Small *et al.*1994) examined three mechanisms of acid resistance, that is,oxidative-arginine dependent and glutamate dependent, and found that all three contribute to the microorganisms overall acid tolerance. Induction of acid tolerance in *E.coli* can enhance its survival in acidic foods (Cheville *et al*, 1996; Layer *et al*, 1995).

(ii) Antibiotic resistance: Sufficient evidence indicates that the bacterium is resistant to most antibiotics.

(iii) Thermal inactivation: Studies on the thermal sensitivity of *E.coli* O157:H7 in ground beef have revealed that the pathogen has no unusual resistance to heat, with D values at 57.2°C,60°C, 62.8°C and 64.3°C of 270,45,24 and 9.6 s respectively. Pasteurization of milk(72°C,16.2s) has also been determined to be an effective treatment that will kill more than 104 *E.coli* O157:H7 cells per ml(D'Aoust et al,1988).Proper heating of foods of animal origin (63°C) is an important critical control

point to ensure activation of *E.coli* O157:H7.

(iv) Inability to grow well: It is important to note that many VTEC strains do not grow well at 44°C, if at all, above 44°C.The minimum growth temperature for *E.coli* O157:H7 under otherwise optimal condition is approximately 8 to 10°C (Buchanan&Bagi, 1994).

(v) Inability to ferment sorbitol within 24 hours: Most but not all O157 VTEC strains do not ferment sorbitol.

(vi) Inability to produce ß-glucoronidase: Most of the O157 VTEC strains will not hydrolyze 4-methyl umbelliferyl-D-glucoronide.

(vii) Inability to produce gas and indole at 44°C: Hence such methods would probably fail to detect VTEC.

(viii) Possession of an attaching and effacing (eae) gene: This property contributes to VTEC to establish its pathogenicity.

(ix) Carriage of a 60-MDa plasmid: *E.coli* O157:H7 isolates associated with human illness harbour a plasmid (pO157) of approximately 60 MD and that contains DNA sequences common to plasmids present in other serotypes of VTEC isolated from patients with Haemorrhagic colitis. It was hypothesized that the plasmid is believed to play a role in the pathogenicity of disease, but its function is unclear.

(x) Expression of an uncommon 5,000 to 8,000 molecular weight outer membrane protein.

Reservoirs and sources of *E.coli* O157:H7

Several reservoirs and sources of *E.coli* O157:H7 have been identified. The association of *E.coli* O157:H7 with undercooked ground beef and raw rice led to investigations of the role of cattle as a reservoir of the pathogens. Several surveys of faecal shedding of *E.coli* O157:H7 produced the following general observations.

- Young animals tend to carry *E.coli* O157:H7 more frequently than adults(Zhao *et al*.,1995)
- Prevalence of faecal excretion varies substantially among positive herds (Zhao *etal*.,1995).
- *E.coli* O157:H7 levels in calf faeces range from less than 10 2 CFU/g to 10 5 CFU/g (Zhao *et al*.,1995)
- Faecal shedding of *E.coli* O157:H7 frequently is intermittent and of short duration, i.e. several weeks to months (Brown *et al*.,1997; Cray & Moon,1995)
- More than one strain of *E.coli* O157:H7 can be isolated from faeces of the same animal or different animals within the same herd (Faith *et al*., 1996; Ming *et al*., 1995).
- Calves have been experimentally infected with *E.coli* O157:H7 (Brown et al.,1997; Cray and Moon,1995).The results revealed that,

1. *E.coli* O157:H7 is not pathogenic to calves. Inoculation with 1010 CFU did not induce significant clinical disease.

2. *E.coli* O157:H7 is continued to the gastrointestinal tract, with the fore stomachs (rumen, reticulum and omasum) and distal sites (distal ileum, proximal caecum, spiral colon and descending colon) being the principal sites of localization.

3. *E.coli* O157:H7 did not form attaching and effacing lesions and did not colonize mucosal surfaces.

The prevalence of *E.coli* O157 in cattle has been reported to range from 0.1 to 16%.The organism has also been isolated from the faeces of geese, sheep, horses, dogs, seagulls, goats and deer. The organism has also been isolated from environmental sources such as cattle, water troughs and soil.

Sources of *E.coli* O157:H7 for cattle have not been clearly identified. Possible sources include contaminated feedstuffs or water,colonized animals in herds,infected wildlife and humans or contaminated facilities and equipment surfaces from contact with faeces.

Transmission of *E.coli* O157:H7

Although a variety of foods have been implicated in *E.coli* O157:H7 associated illness, most outbreaks have been associated with consumption of raw or undercooked foods of bovine origin. *E.coli* O157:H7 infections also were associated with eating other foods, including vegetables, apple cider, cantaloupe, mayonnaise-containing salad dressing and salami. Contact of foods with *E.coli* O157:H7 containing meat or faeces (human or bovine) is a likely source of cross-contamination. Person-to-person transmission (13.2%) and waterborne (4.4%) outbreaks have been documented.

The mechanism of transmission in food chain is not fully understood, but contamination of meat from intestinal contents at slaughter is probably an important factor.

Detection of *E.coli* O157:H7

(i) meat: It is not easy to detect VTEC in raw meats and foods where low levels of *E.coli* may be swamped by high numbers of other bacteria. So far, there are no widely recommended methods for routine food examination. Currently traditional

isolation methods for foods involve enrichment in a selective broth followed by plating on to sorbitol Mac conkey agar with additives. This agar is only suitable for 0157:H7 strains (most but not all 0157 VTEC strains do not ferment sorbitol).The composition of the enrichment broth and plating agar is important if VTEC is to be isolated from contaminated materials and several researches are going on determining the optimum combination of selective agents.

(ii) Stool: Methods used in medical laboratories to detect the organism from stools are more successful, probably the number of VTEC cells present in the stools of someone made ill by the organism is relatively high in comparison to the background flora. For *E.coli* O157 (but not all VTEC serotypes) commercial kits are available for isolation and identification (ELISA methods) and for confirmation of suspect colonies (latex agglutination).Recipes for several effective broths and agars have been published but there is no consensus yet on which is the best. Another technique used to enhance isolation of VTEC from enrichment broths is the use of commercially available immunomagnetic beads coated with specific O157 antiserum. An immunoblotting technique is available for rapid identification of O157 colonies on agar plates.

For epidemiological purpose/surveillance O157 VTEC can be distinguished by phage typing (Ahmed *et al*., 1987) which can be combined with typing of the VT genes to give added discrimination.

Other methods such as plasmid profile analysis, pulsed field gel electrophoresis and multilocus enzyme electrophoresis may further differentiate O157 VTEC and can be applied to VTEC of other serogroups. Restriction fragment length polymorphism analysis of genomic DNA probed with phage ë or the DNA of a VT encoding phage have also been used to differentiate O157 VTEC strains. Thus for surveillance, isolation of *E.coli* O157 should be attempted using readily available methods (e.g.: cefixime tellurite sorbitol Mac conkey Agar) and testing of non-forming sorbitol colonies with 0157 antiserum. Biochemical and serological confirmation tests may also be needed to exclude false positives.VTEC strains other than 0157 do not have biochemical markers that assist in their identification.

The general methods which rely on enrichment followed by plating onto selective agar and biochemical confirmation all at 37°C would be more likely to isolate VTEC. Method developed using cultural and rapid techniques are progressing fast.

Characteristics of the disease

Fortunately infection from *E.coli* 0157 is relatively rare. Its principal symptom, diarrhoea, is also a symptom of other gastrointestinal infections. This has meant that the relatively few *E.coli* O157 have led to be found from among many more infections with this routine symptom.

E.coli O157 infections are associated with a range of illness in humans, although a proportion may be asymptomatic. Where symptoms do occur, the incubation period is 2 to 10 days, with most cases occurring in 3 days.

The range of clinical disease includes:

- Mild diarrhoea, fever, abdominal pain, vomiting
- Hemorrhagic colitis (HC), which consists of inflammation of the large bowel, with severe blood.
- Haemolytic Uraemic syndrome (HUS), a combination of anaemia, acute kidney failure and low platelet count which may be accompanied by fever.
- Thrombotic thrombocytopenic purport (TTP) characterized by fever, via skin and central nervous system involvement, resulting from aggregation of platelets in various organs.

HUS largely affects children and TTP largely affects adults.TTP is a rare syndrome of *E.coli* O157:H7 infection.

Immunity to *E.coli* O157:H7

An infected patient's serological response against surface epitopes of *E.coli* O157:H7 can last from weeks to months. It can be useful for epidemiological studies to determine the serological responses of patients suspected of *E.coli* O157:H7 infection when stool cultures are negative for recovery of *E.coli* O157:H7. However, such studies are limited by the fact that all patients who were *E.coli* O157:H7 culture positive have a demonstrable antibody response. Oral inoculation of calves and steers with 1010 *E.coli* O157:H7 induced prompt and sustained increases in serum antibodies to the O157 antigenic LPS and to a lesser extent to Stx 1(Johnson *et al*., 1996).The serological responses, however, do not correlate with elimination of carriage by cattle or protection of calves against reinfection by the same strain. The ability of *E.coli* O157:H7 to persist in and reinfect cattle that have a strong immune response is likely to contribute to the introduction and persistence of infection in herds.

Prevention and control methods

The prevention of infection requires control measures at all stages of the food chain from agricultural production on the farm, to processing, manufacturing and preparation of foods in both commercial establishments and the domestic environment.

There are insufficient data to recommend specific intervention methods on the farm in order to reduce the incidence of *E.coli* O157:H7 in cattle and other ruminants.

Farms: An important component of Hazard Analysis Critical Control Point (HACCP) application in animal production is reducing the carriage of *E.coli* O157:H7 by animals. Two approaches that have potential are competitive exclusion and vaccination. Competitive exclusion involves the use of microbial cultures that out compete pathogens from colonizing specific niches. This approach uses defined bacterial cultures that can greatly reduce colonization of campylobacter jejuni in poultry (Schoeni and Doyle,1992).

Vaccination: Traditional vaccination approaches are not likely to be successful with *E.coli* O157:H7.Recent observations showed that *E.coli* O157:H7 does not form attaching or effacing lesions or colonize mucosal surfaces of the gastrointestinal tract (Brown *et al*., 1997; Cray and Moon, 1998) and cattle exposed to *E.coli* O157:H7 are not protected from reinfection (Johnson *et al*., 1996).Hence innovative approaches will be needed for vaccines to be effective.

Slaughterhouse: Like other E.coli, it is assumed that the ultimate source of *E.coli* O157:H7 in carcasses is faecal contamination during animal production and slaughter operations. Faecal contamination is associated primarily with contamination of the carcass during hide removal and spreading of contamination to other carcasses by equipment and workers hands (Dickson & Anderson, 1992).

Quality assurance programmes in slaughter houses should stress the need to minimize the faecal contamination of carcasses and to chill meat rapidly. Screening of raw meats for VTEC is not an effective control mechanism because isolation rates from raw beef are low and the organism has been found in the faeces of a small proportion of healthy cattle, so currently it is unlikely that it can be eliminated at source. Simple and reliable methods suitable for routine VTEC detection in foods are not widely available. Similarly, because of the low contamination rate of VTEC in other foods, routine screening specifically for this organism is unlikely to be worthwhile or successful.

As screening can never detect all contaminated loss, it is a poor control procedure. Adequate cooking of meat is the only sure way of eliminating the danger of VTEC infection from this source.

For most food manufacturers, surveillance of raw and in-process materials, finished products and the manufacturing environment should be based on needs identified by HACCP evaluation, and the end product specification. Monitoring trends of indicator organisms and standard plate count indicate deviations from quality standards.

Food processing: *E.coli* O157:H7 can be controlled readily through traditional thermal processing techniques.

Recommendations to reduce the risk of acquiring an *E.coli* O157:H7 infection.

1. Cook ground beef thoroughly (minimum 160°F) before eating.
2. Drink only pasteurized milk and apple juice.
3. Wash fresh fruits and vegetables thoroughly before eating.
4. Wash hands thoroughly after handling animals, particularly cattle,deer,goats or dogs.
5. Wash hands thoroughly after changing diapers or after providing care to children or adults suffering from a diarrhoeal disease.
6. Do not use fresh manure from ruminants to fertilize vegetables or fruits.
7. Avoid swimming in lakes or ponds used by cattle and drinking surface water that has not been properly treated to eliminate pathogens.

Conclusion and recommendations

The serious nature of the symptoms of haemorrhagic colitis and HUS and the apparent low infectious dose (<100 cells) of *E.coli* O157:H7 places this food borne pathogen a most serious of known food borne pathogens.

Persuasive evidence suggests that healthy cattle are a reservoir of O157 and they can enter the food chain to provide a source of exposure for humans. A possible route of transmission of O157 VTEC may involve infections initially in calves that shed their organism into faecal slurry that may be used on grazing grass. This provides potential for infection of other animals from which the organism may contaminate milk or carcasses at slaughter. Possible sources of VTEC in healthy animals other

than cattle and a wider range of foodstuffs require further investigation.

Many features of *E.coli* O157:H7 strains remain poorly understood. It includes:

(i) Role of virulent genes in the animal,
(ii) Mechanism of evolution of the organism,
(iii) The progress of individual cases of *E.coli* O157:H7 infection, and
(iv) The difference in incidence of infection in different geographical areas.

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Dozen peacocks die mysteriously in UP village

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TEDUI: Over a dozen peacocks have died mysteriously at a village in Uttar Pradesh's Allahabad District, officials have confirmed. The mysterious deaths were reported from Tedui village.

"More than 50 peacocks were living in the vicinity, but for the last two to three days, more than a dozen have died suddenly and the reason for their death is not clear," said Raju, a local.

Veterinary officials have collected samples from the dead birds to ascertain the cause of death.

"So far we have done an autopsy of three peacocks. We are now sending some of the bodies to a laboratory in Lucknow to ascertain the cause of death. We suspect that the deaths could have been because of poisoning," said D R Ram, a veterinary doctor.

The blue peacock, scientifically known as Pavo Cristatus, is regarded as one of the most beautiful birds throughout the world and is provided with adequate safety in India, under the Indian Wildlife Protection Act, 1972.

The poaching of this swan-sized bird, with a long and slender neck, is a punishable offence. The peacock is India's national bird and its feathers, adorned by the mythical Hindu god-king Krishna, are considered auspicious.

Besides natural factors, the destruction of habitats, poaching and contamination of food, are severely hampering the growth of the peacock population.

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