Escherichia coli O157:H7 - An Emerging Pathogen in foods of Animal Origin

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Abstract

Escherichia coli O157:H7 is an emerging public health concern in most countries of the world. *E. coli* O157:H7 was known to be a human pathogen for nearly 24 years. EHEC O157 infection is estimated to be the fourth most costly food borne disease in Canada and USA, not counting the cost of possible litigation. *E. coli* O157:H7 and Salmonella are the leading causes of produce related outbreaks, accounting for 20 and 30% respectively. The authority of the Federal Meat Inspection Act, FSIS (Food Safety and Inspection Service) declared Escherichia coli O157:H7, an adulterant in raw ground beef and enforced "zero tolerance" (USDA-FSIS, 17 December 1998). Because of the severity of these illnesses and the apparent low infective dose (less than 10 cells), *Escherichia coli* O157:H7 is considered one of the most serious of known food borne pathogens. *Escherichia coli* O157:H7 is mainly pathogenic to human but in cattle and other animals, it did not induce any clinical disease except diarrhea. So, these animals act as carriers to *Escherichia coli* O157:H7. The majority transmission is through eating of undercooked contaminated ground meat and consumption of raw milk, raw vegetables, fruits contaminated by water, cheese, curd and also through consumption of sprouts, lettuce and juice. The conventional isolation procedure includes growth in enrichment broth like modified EC (*E. coli*) broth or modified tryptic soy broth (mTSB) Since the infection primarily occurs via faeco-oral route, the preventive measures include food hygiene measures like proper cooking of meat, consumption of pasteurized milk, washing fruits and vegetables especially those to be eaten raw and drinking chlorine treated water and personnel hygiene measures like washing hands after toilet visits.

Keywords: Food borne pathogen, Enteritis, Meat, Animal products, Zoonosis, Outbreak, Public Health.

Introduction

Escherichia coli O157:H7 is an emerging public health concern in most countries of the world. This was first recognized as a cause of illness in 1982 during an outbreak of severe bloody diarrhea traced to consumption of hamburgers at common chains of fast-food restaurants (Riley et. al., 1983). Since then, infections have been reported from more than 30 countries on six continents. In India, for the first time the occurrence of *E. coli* O157:H7 was reported in buffalo meat kebabs, sausages, buffalo milk, cow milk and khoa sweet in 1966 (Singh et. al., 1996).

E. coli is an important member of the coliform group. Based on the pathogenecity and variation in bio-chemical characteristics, *E.coli* has been classified into 6 categories, viz. enterohaemorrhagic (EHEC), enterotoxigenic (ETEC), enteropathogenic (EPEC), enteroinvasive (EIEC), enteroaggregative (EaggEC) and diffusely adherent (DEAC), of which the EHEC is considered as most important one. Although, most strains of *E. coli* are harmless and normally inhabit the intestines of healthy animals and humans, E. coli O157:H7 is most notorious serogroup of Verotoxigenic/ Shigatoxigenic *Escherichia coli* (VTEC/STEC) and belongs to a sub-group of VTEC that is associated with human beings, and referred to as enterohaemorrhagic E. coli (EHEC). Relatively, recent acquisition of VT-converting phages is believed to be largely responsible for the recent emergence of *E. coli* O157:H7 as a new pathogenic mutant from more mildly pathogenic O55:H7 and O127:H6 progenitors (Whittam, 1995). The E. coli O157:H7 (EHEC) strain produces two types of toxins namely Shiga toxin 1 (Stx-1) and / or Shiga toxin 2 (Stx-2), which have been also referred as Verotoxin 1 (VT-1) and Verotoxin 2 (VT-2) respectively, responsible for food borne illness. The ability to produce shiga toxin was acquired from a

bacteriophage presumably directly or indirectly from Shigella.

Prevalence of *E. coli* O157:H7 and Zoonotic importance

E. coli O157:H7 was known to be a human pathogen for nearly 24 years (Riley et. al., 1983). In 1993, the Council of State and Territorial Epidemiologists recommended that *E. coli* O157:H7 be a nationally reportable disease and that clinical laboratories screen atleast all bloody stools for this pathogen (CSTE, 1993).

Experts estimate that 76 million cases of human diseases, 325,000 hospital admissions and 5,000 mortalities are caused annually in US by consumption of contaminated food. According to the Centers for Disease Control and Prevention nearly 74,000 cases and 61 deaths annually are attributable to this pathogen in USA (CDC, 2003) due to consumption of contaminated beef (Mead et. al., 1999). Pennington Group (1997) reported that 21 people died and more than 500 fell ill due to an outbreak of Escherichia coli O157:H7 in Central Scotland at the end of 1986. This was among one of the world's worst food borne outbreaks in terms of morbidity and mortality in humans. Approximately 52% of recorded human disease outbreaks have been associated with bovine products (Griffin and Tauxe, 1991).

EHEC O157 infection is estimated to be the fourth most costly food borne disease in Canada and USA (Todd, 1989), not counting the cost of possible litigation. *E. coli* O157:H7 and Salmonella are the leading causes of produce related outbreaks, accounting for 20 and 30% respectively (Olsen et. al., 2000).

More than 500 laboratories confirmed infections with *E. coli* O157:H7 and 4 associated deaths occurred in 4 states of U.S.A which resulted from the consumption of hamburgers from one restaurant chain (CDC, 1993). In 1994, an outbreak of Escherichia coli O157:H7 infection linked to commercially distributed dry-cured salami product in Washington and California was reported (MMWR, 1995) which led to voluntary recall of 10,000 pounds of implicated product and suspended the sale of all the products until the source of contamination was determined. An outbreak of *E. coli* O157:H7 infection occurred in U.K. following consumption of commercial Yogurt (Morgan et. al., 1993). Also contamination of household supply water with sewage water near the sites of water main breaks results in water borne outbreak of *E. coli* O157:H7 infection (David et. al., 1992).

The infection by *E. coli* O157:H7 have been reported with increasing frequency from all parts of the world in the form of food poisoning outbreaks (Jo et. al., 2004). Considering the nature and severity of outbreaks, WHO Consultation Committee recommended enhanced investigation, reporting and strict surveillance of shiga toxin producing *E. coli* (STEC) and HC-HUS (WHO, 1997). The authority of the Federal Meat Inspection Act, FSIS (Food Safety and Inspection Service) declared Escherichia coli O157:H7, an adulterant in raw ground beef and enforced "zero tolerance" (USDA-FSIS, 17 December 1998). Because of the severity of these illnesses and the apparent low infective dose (less than 10 cells, Bach et. al., 2002), *Escherichia coli* O157:H7 is considered one of the most serious of known food borne pathogens (Blanco et. al., 2003).

Number of human infections peaks during the summer months (Bach et. al., 2002) and this may be due to more frequent consumption of ground beef and more frequent contact with domestic animals. Houseflies and blow flies can carry relatively high concentrations of potentially virulent Escherichia coli O157:H7 during summer (Keen et. al., 2006). The identification of different vehicles for transmission of *Escherichia coli* O157:H7 confirms that *Escherichia coli* O157:H7 can survive in conditions long considered to be inhospitable to enteric pathogens, such as low PH and high salinity (Leyer et. al., 1995).

Human to human and animal to human contact have been implicated in the transmission of the disease (Duffy, 2003). E. coli O157:H7 has been isolated from a wide variety of hosts, specially cattle, sheep, goat, pig, poultry, dog, horses, deer, wild birds, flies (Hancock et. al., 1998).

The occurrence of *E. coli* O157 and *E. coli* O157:H7 multiple antibiotic resistant profiles may show a risk for Public Health and food safety as well as animal health and production (Ulukanli et. al., 2006). The distribution of and increase in the multiresistant VTEC in animal species underline the necessity of minimizing the use of antibiotics in animal production (Von Muffling et. al., 2007).

High risk groups:

The highest attack rate was among children less than 5 yrs of age but cases were reported in persons of 11 months to 78 yrs of age also (Ostroff et. al., 1989). Females have been reported to be at a significantly greater risk of developing haemolytic anaemia after infection with *Escherichia coli* O157:H7 than males (Rowe et. al., 1991). Cimolai et. al. (1990) also reported that males have a reduced risk of Escherichia coli O157:H7 colitis progressing to HUS than females.

Outbreaks of *E. coli* O157:H7 have involved communities (Swerdlow et. al., 1992) and institutions such as nursing homes (Carter et. al., 1987), schools and day care facilities (Belongia et. al., 1993). The largest number of *Escherichia coli* O157:H7 isolates were obtained from children of 1-4 years age and adults of 60-69 years age (Slutsker et. al. 1997).

Pathogenesis in Human

Escherichia coli O157:H7 is mainly pathogenic to human but in cattle and other animals, it did not induce any clinical disease except diarrhea (Brown et. al., 1997). So, these animals act as carriers to Escherichia coli O157:H7. The pathogenicity of *Escherichia coli* O157:H7 is associated with a number of virulence factors, including shiga toxins (Stx1 and Stx2; encoded by the stx1 and stx2 genes), intimin (encoded by the eae gene) and the enterohaemolysin (encoded by the hlyA gene) (Karch et. al., 1998). *Escherichia coli* O157:H7 strains carrying stx2 gene along with enterohaemolysin gene are potentially dangerous to human health (Manna et. al., 2006). Stx2 producing strains appear to be more commonly responsible for serious complications such as HUS than those only Stx1 producing (Kleanthous et. al., 1990).

Pathogenicity of *Escherichia coli* O157:H7 is encoded by a variety of plasmid, bacteriophage and chromosomal genes. EHEC contain virulence plasmids that promote non-intimate attachment and pathogenicity island encoding both attachment and the signaling apparatus to induce attaching effacement of the mammalian enterocyte (Saunders et. al., 1999). Following the ingestion of O157 isolate, organisms adhere to and colonize the bowel mucosa. This may be mediated in part by the eae gene (Donnenberg et. al., 1993). Shiga toxins bind to receptors on the bowel mucosa and are elaborated and translocated into the cell interior and inactivate ribosomal RNA leading to the inhibition of protein synthesis in cells expressing glycolipid G3b (globotriaosylceramide) and eventually causes death of host cells (Mead and Griffin, 1998). High levels of G3b are found in human kidney which is 1000 times more sensitive to the cytotoxic action of stx2 than that of stx1 (Louise and Obrig, 1995).

Blood released due to mucosal damage is lysed by enterohaemolysin liberating heme and haemoglobin which facilitates rapid multiplication of the organism (Nataro and Kaper, 1998). As multiplication takes place, further toxin production occurs, causing greater damage, releasing increasing amounts of blood and resulting in growth stimulation (Law and Kelly, 1995).

Transmission of Disease

Transmission usually occurs through consumption of undercooked or contaminated foods of bovine origin, faecal contamination of other food products or direct contact with infected animals. Cattle and sheep are usually recoginsed as the principal reservoirs responsible for the proliferation of *E. coli* O157:H7. Since infection occurs via faecal-oral route, the numbers shed in faeces and susceptibility of the host ultimately determines transmission of the organism.

Symptoms in Human

Different human diseases, from mild diarrhea to hemorrhagic colitis, haemolytic uraemic syndrome and Thrombocytic Thrombocytopaenic Purpura, can be caused by the O157:H7 serotype of Shiga toxin producing *E.coli* (STEC), especially among children, the elderly and others with under developed immunity (Gage et. al., 2001).

The reported clinical signs and symptoms of *E.coli* O157:H7 infections include bloody or non-bloody diarrhoea, abdominal cramps and little or no fever is present and the illness resolves in 5-10 days (Tarr, 1995). Abdominal tenderness associated with *E.coli* O157:H7 infection may contribute to misdiagnoses, such as appendicitis or intusussception and may result in unnecessary surgical procedures (Griffin, 1995). The disease mainly produces three types of syndromes, viz. haemorrhagic colitis (HC), haemolytic uraemic syndrome (HUS) and thrombocytic thrombocytopaenic purpura (TTP).

Haemorrhagic Colitis (HC): HC caused by EHEC O157:H7 is characterized by severe abdominal cramps, bloody stools, little or no fever and evidence of colonic mucosal edema (Griffin and Tauxe, 1991). Right sided colonic inflammation by barium enema or colonoscopy in patients with *E.coli* O157:H7 infection was also observed (Pavia et. al., 1990).

Haemorrhagic Uraemic Syndrome (HUS):The estimated annual rate of HUS in Argentina is 9.2 per 100,000 in children under 5 years of age with more than 7000 cases of HUS reported since 1965 (CNSAP, 1995). 2 to 10% of patients with VTEC infection may develop HUS upto 3 weeks after the onset of diarrhoea (Karmali et. al.,1985). Mortality rates following development of VTEC associated HUS in developed countries are approximately 2 to 10% (Pickering et. al., 1994). HUS is one of the most common causes of acute renal failure in children and is characterized by microangiopathic haemolytic anaemia, oliguric renal failure, thrombocytopenia and CNS symptoms (Robson et. al., 1993). Death in the acute phase is due to renal failure, severe hypertension, myocarditis or neurological disease. Among survivors of HUS, 10% suffer chronic renal failure and another 40% have renal insufficiency or other persistent sequelae (Pickering et. al., 1994).

Thrombocytic Thrombocytopaenic Purpura (TTP): This is considered to be a manifestation of HUS, in elderly, where renal failure is normally mild but neurological involvement is greater with a mortality rate as high as 50% (Griffin, 1995).

Food borne infection

The majority transmission is through eating of undercooked contaminated ground meat and consumption of raw milk, raw vegetables, fruits

contaminated by water, cheese, curd and also through consumption of sprouts, lettuce and juice. Hence the prevalence and occurrence of *E.coli* O157:H7 in foods of animal origin are detailed here under.

Beef: Contaminated undercooked ground beef and hamburgers have been the most frequently identified vehicle of *E.coli* O157:H7 in human disease outbreaks (Griffin and Tauxe, 1991). Presence of Shiga toxin producing *Escherichia coli* O157 in beef and milk is of serious public health concern. Cattle faeces were the probable sources of contamination. Isolation of the pathogen from pasteurized milk indicates inadequate pasteurization or post-pasteurization contamination (Armstrong et. al., 1996). The incidence of Escherichia coli O157:H7 in beef was reported as 31% in Canada (Doyle and Schoeni, 1987).

E. coli O157:H7 has been reported in refrigerated ground meat, refrigerated long pork sausages and frozen hamburgers (Alexandre et. al., 2001) precooked meat patties (Belongia et. al., 1991). *Escherichia coli* O157:H7 appears to bind to collagen fibrils on beef tissues, techniques with the bacterial collagen interaction may be effective in removing the organisms attached to beef carcass surfaces. 10% TSP (Tri Sodium Phosphate) treatment was more effective against E.coli strains (Fratamico et. al., 1996).

In several recent surveys, researchers found that over 70% of cattle hides may be contaminated with *Escherichia coli* O157:H7 at the beginning of the slaughter process (Barkocy-Gallagher et. al., 2003). Cattle and their faeces have been considered as the primary source of VTEC and the reported incidence of E.coli O157 in cattle faeces was 62% (Jackson et. al., 1998) and higher frequency in younger animals (Desmarchelier and Grau, 1997).

Calves: *E. coli* are isolated more frequently from calves less than 3 months old than from adult cows/ buffaloes and more frequently from diarrheic calves than from normal calves (Heuvelink et. al., 1998). *Escherichia coli* O157:H7 is not pathogenic to calves even with high inoculum (1010 cfu), as it did not induce the significant clinical disease, in experimental trials (Brown et. al., 1997).

Milk and Milk Products: Milk is one of the most common sources of *Escherichia coli* O157:H7 infection and it is mainly due to faecal contamination (Armstrong et. al., 1996). The frequent epidemiologic evidence of milk as a source of human O157:H7 infection suggests the role of mammary gland, as a potent source of infection (Wells et. al., 1991). *E. coli* O157:H7 was isolated from commercially distributed raw milk (William et. al., 1997), raw milk stored in bulk tanks (Padhye and Doyle, 1991), pasteurized milk (Upton and Coia, 1994) and also from cheese (Mora et. al., 2007). In U.S. upto 10% raw milk samples from bulk tanks on farms were positive for *E. coli* O157:H7 (Padhye and Doyle, 1991).

In India, Suresh (1999) isolated this organism from kulfi samples. Studies with artificially contaminated cow's milk have demonstrated that addition of components of the Lacto-Peroxides System (LPS) resulted in inhibition and inactivation of O157 VTEC (Heuvelink et. al., 1998).

Sheep: Sheep could be an equally important reservoir of O157:H7 (Kudva et. al. 1996). Sidjabat-Tambunan et. al. (1998) reported that majority of Escherichia coli O157:H7 isolates (78.6%) isolated from mutton carcasses were found to produce enterohaemolysin and there is a predominance of isolates producing VT1 and VT2 in winter, with nearly 66% of the isolates producing these verocytotoxins, while in summer the majority of the isolates produced VT1 only. Escherichia coli O157:H7 was also isolated from 2% of lamb meat samples (Doyle and Schoeni, 1987).

Chapman (2000) reported that the incidence of *Escherichia coli* O157:H7 isolates was higher in lamb products than in beef products. Ulukanli et. al. (2006) isolated *Escherichia coli* O157:H7 from sheep raw milk samples using conventional methods and confirmed using biochemical tests.

Goat: *Escherichia coli* O157:H7 has been isolated from goat's milk by Bielaszewska et. al. (1997). Dontorou et. al. (2004) reported a rare isolation of *Escherichia coli* O157:H7 strain from goat faeces.

Pork and Poultry Products: Food animals like pigs and chicken also appear to be reservoirs of this organism (Beutin et. al., 1993). Heuvelink et. al., (1999) isolated *Escherichia coli* O157:H7 from raw minced pork, other raw pork products and cooked or fermented, ready to eat meats like sausages. STEC O157 were isolated from retail beef, poultry and pork samples in the United States and Canada (Doyle and Schoeni, 1987).

Escherichia coli O157:H7 was also isolated from cheese and turkey sandwiches (Ryan et. al., 1986), ham and turkey sandwiches (Carter et. al., 1987), hamburgers, salad components, sea eel susli and soup (Bettelheim, 1997) and dry cured salami (MMWR, 1995).

Egg and egg products: Brackett (1988) reported *Escherichia coli* O157:H7 to be present in egg and egg products like steamed eggs and scrambled eggs.

Deer: *E. coli* O157:H7 was isolated in Oregon from persons, who had consumed venison jerky (Sanchez et. al., 2007) and undercooked venison (Rabatsky-Ehr et. al., 2002). Sargeant et. al. (1999) isolated *Escherichia coli* O157:H7 from deer faeces which was collected in cattle pastures which indicates that cattle are the source of infection.

Dog: *Escherichia coli* O157:H7 phage type 4 has been isolated from dog faeces (Trevena et. al., 1996).

Other Products: *Escherichia coli* O157:H7 was

associated with apple cider (Besser et. al., 1993), lettuce (Ackers et. al., 1998), melon and spinach (USFDA, 2006), radish sprouts (WHO, 1996), potatoes and alfa-alfa sprouts (Heuvelink et. al., 1999). Handling of raw potatoes that were packed in peat that may have been contaminated with calf manure was linked to another outbreak of *Escherichia coli* O157:H7 infections (Morgan et. al., 1988).

Diagnosis

The conventional isolation procedure includes growth in enrichment broth like modified EC (*E. coli*) broth or modified tryptic soy broth (mTSB), both supplemented with novobiocin or acriflavin (Doyle and Schoeni, 1987) at 37^{0}C for 18-24 hr to increase the ratio of *E. coli* O157:H7 to competitor organisms and plating on selective medium, CT-SMAC i.e. Sorbitol MacConkey agar supplemented with Cefixime and Tellurite (Zadik et. al., 1993) for 24-48 hr at 37^{0}C. Strains of *E. coli* O157:H7 do not ferment sorbitol and produce colourless colonies where as other serotypes of *E. coli* ferment sorbitol and produce pink colonies. So, this medium is widely used for isolation of *E. coli* O157:H7.

Prevention and Control

Since the infection primarily occurs via faeco-oral route, the preventive measures include food hygiene measures like proper cooking of meat, consumption of pasteurized milk, washing fruits and vegetables especially those to be eaten raw and drinking chlorine treated water and personnel hygiene measures like washing hands after toilet visits.

Therefore considering the severity of infections, *Escherichia coli* O157:H7 should be given considerable preference in diagnosing the infections and eliminating the transmission through livestock products.

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