



Public Health Significance of Verocytotoxigenic *Escherichia coli*: An Overview

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10.18805/ag.R-2680

ABSTRACT

Meat is a global vital protein source catering to diverse diets. By 2050, world's population may rise to 9 billion and the demand for meat will exceed supply, necessitating a doubling of meat production. Nutritionally rich composition makes meat, a fertile ground for numerous microbes, leading to spoilage and foodborne illnesses, impacting human health. External factors like poor hygiene, inadequate post-production storage, mishandling and more contribute to meat contamination. The zoonotic strains of *E. coli* can cause severe food poisoning, with over 700 identified serotypes, hence it is crucial to identify, monitor and control *E. coli* to ensure food safety. Among *E. coli* pathotypes, Verocytotoxigenic *E. coli* (VTEC) stands out as major cause of food poisoning. The VTEC toxin (Shiga toxin), can induce mild diarrhea to severe form of illness like Hemorrhagic Uremic Syndrome and Hemorrhagic Colitis, posing a severe public health risk. The Cattle is a primary reservoir for VTEC, which, by faecal shedding, can pollute food, water and the environment. Furthermore, *E. coli* can spread through contaminated meat, raw milk and milk products. This bacterium is commonly found in the immediate surroundings of animal reservoir, including soil, grass and manure, leading to potential contamination of milk. *E. coli* infections not only impact humans but also animals, with cattle, sheep and poultry acting as reservoirs for different *E. coli* strains. Cattle populations frequently harbor *E. coli* O157:H7 asymptomatic carriers, contributing to faecal contamination of food and water sources. Educating the public about the risks associated with improper meat handling and contamination is essential to reduce foodborne illnesses. Monitoring and surveillance of meat quality in local markets, as are crucial steps towards ensuring food safety. This underscores the importance of understanding and managing *E. coli* contamination in meat to safeguard public health and ensure the safety of meat products in the global food supply chain.

Key words: *E. coli*, Food safety, VTEC.

Meat is an important source of protein for people who barely get food, to those who live in high maintenance societies. Many cuisines like Mughlai, Indian, Thai and Mexican, use meat as a primary ingredient. Though beef and mutton are the most popular meats, consumption of chicken is much higher than other meat foods due to its readily availability and lower cost (Rudhy, 2017). The human population of the world is rapidly increasing and it is predicted that the demand for meat will surpass the supply and meat production will have to be doubled by 2050 to satisfy the human need.

Meat being rich in minerals, moisture and nitrogenous chemicals, it is most suitable medium for growth and multiplication of different microbes, resulting in its spoilage and thereby acts as source for food borne illnesses. The lack of hygiene, improper post-production storage, faulty handling of food products etc. are also responsible for contamination and spoilage of meat and adverse effect on the human health (Stringer *et al.*, 1969; Thanigaivel and Anandhan, 2015).

Various bacteria viz, *E. coli*, *Enterobacter aerogens*, *Salmonella* spp., *Staphylococcus* spp., *Pseudomonas* spp., etc., have been detected in uncooked meats. *E. coli* infections are zoonotic in nature and can cause serious food poisoning in human beings. Together with other pathogens *E. coli* is responsible for nearly 76 million incidences of food-borne illness in the United States (CDC, 2005).

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How to cite this article: Bairi, N.S., Savalia, C.V. and Ram, N.S. (2024). Public Health Significance of Verocytotoxigenic *Escherichia coli*: An Overview. Agricultural Reviews. DOI: 10.18805/ag.R-2680.

Submitted: 06-11-2023 **Accepted:** 03-06-2024 **Online:** 24-07-2024

Description of *Escherichia coli*

German bacteriologist Theoder Escherich discovered *E. coli*, originally known as *Bacterium coli commune*, in 1885 (Wasteson, 2002). *Escherichia coli* is mesophilic, facultative anaerobic, straight rod-shaped, lactose-fermenting, motile bacteria measuring 1.1 to 1.5 mm in diameter and 2.0 to 6.0 mm in length. It forms essential part of the intestinal flora of a healthy host. It belongs to the Enterobacteriaceae family, which also includes many other genera like *Shigella*, *Yersinia* and *Salmonella*. They convert

Hemolytic Uremic Syndrome (HUS), Hemorrhagic Colitis (HC), Thrombotic Thrombocytopenic Purpura (TTP), acute renal failure, microangiopathic and hemolytic anemia posing significant burden on public health, world over. *E. coli* O157:H7, one of several serologically different strains of VTEC, is the most common cause of VTEC infections. (Pennington, 2010, Bai *et al.*, 2015; Hussien *et al.*, 2019).

The *E. coli* infections are spread by the fecal-oral route from contaminated food, water, animals and the environment. Ruminants are considered as chief reservoir of VTEC, shedding microbes in their faeces. It has been reported that majority of food borne illnesses found linked to under-cooked ground beef and unpasteurized milk (Lior, 1994, Caprioli *et al.*, 2005, Erickson and Doyle, 2007, Buncic *et al.*, 2014; Pires *et al.*, 2019).

Total 207 VTEC serotypes are identified from cattle and 160 from human being and 150 serotypes are frequently shared between humans and animals, indicating the possibility of the disease being spread by animals and their products (Lior, 1994; WHO, 1995). Therefore, it is crucial to know its occurrence, persistence and dependable techniques for the early identification and suppression of VTEC, to ensure product quality.

Despite the fact that, refrigeration is often thought to be effective in slowing down the development and multiplication or inhibiting the survival of bacteria, the VTEC O157:H7 is observed to survive better in acid environment at 4°C in fermented dry sausage (Conner and Kotrola, 1995).

Verocytotoxin-producing *Escherichia coli*

Konowalchuk *et al.* (1977), identified verotoxic *E. coli*, which was able to produce toxins having significant and irreversible impact on Vero cells. Verotoxins (VT) were encoded on a bacteriophage in *E. coli* in the 1980s (O'Brien *et al.*, 1984). Further research on VTs revealed two main toxin types, VT1 and VT2, which share 55% DNA and with different immunological expression, as VT2 is not cross reactive. Shiga toxin (Stx), which is produced by *Shigella dysenteriae* type 1 and VT1 were genetically and immunologically related. Despite these variations, the functions of the VT1, VT2 and Stx genes are similar and each of these genes is genetically organized in an operon structure with two genes that code for the A and B - subunits of the toxin and cell receptor binding, respectively. As a result, the nomenclature systems SLT (shiga like toxin), Stx (shiga toxin) and VT (verotoxin) have been used interchangeably. Thus, *E. coli* that produces verotoxins (VTEC) is also known as *E. coli* that produces shiga-like toxin (SLTEC), shiga toxin-producing *E. coli*, or STEC.

Escherichia coli O157:H7 firstly recognized in 1982 as a serious animal-borne human disease, when a hemorrhagic colitis outbreak in the US was linked to hamburger consumption (Ngwa *et al.*, 2013). Since then, this infection has been associated to over 200 cases in Scotland, Japan, Canada and the UK (Adamu *et al.*, 2014). Human infections with STEC have also been recorded in

Latin America, India and other countries, even though majority of sporadic cases and outbreaks have been reported from wealthy nations (Qadri *et al.*, 2005).

The VTEC produce a toxin inducing illness from mild diarrhea to extremely severe intestinal inflammation. The morbidity and fatality rates brought on by a number of previous STEC outbreaks have demonstrated a serious threat to public health. The ideal temperature for STEC growth is 37°C and, at 70°C they get destroyed (Rehman *et al.*, 2013).

Epidemiology of *Escherichia coli*

Escherichia coli is a regular inhabitant of the large intestine in humans and warm-blooded animals. The spread of *E. coli* through raw milk and dairy products results from faecal contamination during the milking process and from poor hygiene practices. It primarily spreads by the consumption of contaminated vegetables, unpasteurized milk and raw or undercooked ground beef (Lara *et al.*, 2016).

Septicemia and diarrhea are caused by *E. coli* in cattle as well as other animals such piglets, children, foals and lambs. Cystitis and other urogenital infections can affect both cats and dogs. STEC O157: H7 is the STEC family serogroup that has historically received the most reports. Some non-O157 serogroups, like O26, O91, O103, O104 and O111, have been found in humans. The majority of STEC infections happen during the summer. Children under age of five had the highest attack rate of 10, but human cases were also reported between the ages of 11 months and 78 years (Ostroff *et al.*, 1998).

Nazir *et al.* (2005) reported that *E. coli* infection was more common in diarrheal calves (13.71%) than in non-diarrheic ones (9.1%). *Escherichia coli* O157:H7 outbreaks associated with food poisoning have become more frequent in recent years.

Pathogenesis

The pathogenic strains of *E. coli* cause gastrointestinal illness in healthy humans and animals, when ingested. Most *E. coli* strains are commensals of normal flora of GIT or opportunistic pathogen capable of eliciting severe illnesses including the death.

Escherichia coli which are pathogenic have different virulence factors that allow them to colonize in the small intestines of the host, avoiding the immune response and stimulating the deleterious inflammatory response to produce diarrhea (Younis *et al.*, 2009). Fimbriae and Intimin, as well as the Exotoxins - heat-labile enterotoxin (LT), heat-stable enterotoxins (STa and STb) and verotoxins (VT), are antigens of colonization or adherence as well as virulence factors. The *E. coli* pathotypes that have survived and developed from the most effective combinations of virulence factors, can infect healthy people (Kaper *et al.*, 2004).

Verotoxins (VT1 and VT2), the *eae* gene that encodes Intimin and other virulence factors are all linked to the pathogenicity of verotoxigenic VTEC, which causes

hemolytic uremic syndrome by attaching and effacing the organism to gut epithelial cells.

Human illness

In human being, the virulent strains of *E. coli* cause meningitis in newborns, gastroenteritis and urinary tract infections, including perforation and intestinal necrosis leading to three types of syndromes, viz. Hemorrhagic Colitis, Hemorrhagic Uremic Syndrome and Thrombotic Thrombocytopenic Purpura (Kiranmayi *et al.*, 2010).

From the pathogenic VTEC recovered from human, *Vtx1*, *Vtx2*, *Vtx2c* and *eae* are the main virulence factors recognized. Bovines are thought to be reservoirs for *E. coli* O157:H7 outbreaks linked to food poisoning. The pathogen infects a person at a relatively low level of 10-100 cells. Ground beef use has been linked to numerous human *E. coli* O157:H7 outbreaks (Soderlund *et al.*, 2012).

Diseases in animals

Callaway *et al.* (2009) surveyed cattle population and reported that up to 30% were asymptomatic carriers of *E. coli* O157:H7. The experimental inoculation tests with the bacteria resulted that they were most abundant in the caecum, colon and rectum of cattle, which pose faecal contamination of food, water and environment. A noticeable clustering of human cases was encountered in places with the highest cattle density and there is a significant correlation between the number of human cases and beef cattle population. Non-O157 STEC are more common in minced beef products, than O157 STEC.

The intestinal tract of sheep is considered to be a major reservoir site of STEC. One study conducted in the UK, reported that *E. coli* O157: H7 contamination was more common in raw lamb meat products compared to beef (Caro *et al.*, 2007).

Poultry and its products are associated with *E. coli* infection in most of the countries in the world. Swollen head syndrome in poultry is an acute respiratory condition caused by STEC (Doane *et al.*, 2007).

Incidence from meat

Ulukanli *et al.* (2006) collected 80 cooked meat samples from restaurants in Kars for the detection of *Escherichia coli* O157 using SMAC and found 37 samples to be presumptive for *E. coli* O157.

Varela-Hernandez *et al.* (2007) analyzed 258 beef carcasses from slaughter plant in Mexico and found 20 samples were positive for *E. coli* O157.

Yadav *et al.* (2007) conducted a study in Anand, Gujarat, India, using 100 samples of mutton and reported that 49 samples were positive for *E. coli*.

Koitaishi *et al.* (2008) studied 58 beef samples in China and reported that 31(58%) samples were positive for *E. coli* O157.

Abong'o and Momba (2009) worked on *E. coli* O157:H7 in 45 samples of biltong, cold meat, minced meat and polony, which were sold in the South African. Of total 180

beef and meat products inspected, 5 (2.8%) were found contained with *E. coli* O157:H7.

Akond *et al.* (2009) screened total 250 meat samples and recorded 45 (58%) positive for *E. coli*.

Tahamtan *et al.* (2010) showed *E. coli* prevalence of 34.76% in the recto-anal-mucosal swabs of calves, using modified Tryptic Soya broth as selective enrichment and streaking on EMB agar.

Chavhan *et al.* (2012) investigated the pathogenic characteristics of *E. coli* isolated from broiler meaty, 35 dressed raw meat samples, together with 17 samples from intestines, were obtained from meat market, Nagpur, India. A total of 37 (71.1%) *E. coli* isolates were recovered, which included 20 (57.1%) raw meat and 17 (100%) intestinal samples of chickens.

El-Jakee *et al.* (2009) found 11.2% prevalence in cattle, buffaloes and chicken meat using Mac Conkey agar for primary isolation and EMB agar for selective isolation.

Momtaz and Jamshidi (2013) cultured *E. coli* from 422 chicken meat samples collected from 5 Iranian towns and 146 (34.59%) samples were found positive for *E. coli*.

Elbayoumi *et al.* (2018) studied 210 poultry meat samples which included chicken thigh, chicken breast and chicken products to identify *E. coli*. The results yielded *E. coli* in 14.3% chicken breast and 20% chicken thigh samples.

Sethulekshmi *et al.* (2018) examined 758 samples from Kerala. The samples comprised of raw milk (135), pasteurized milk (100), beef (132), buffalo meat (130), chevon (104), beef kheema (115) and beef sausage (42). The occurrence of *E. coli* was 19.26%, 41.6%, 16.92%, 28.85% and 41.74%, respectively.

Bedasa *et al.* (2018) evaluated 200 samples of milk, meat and their products. Out of which 40 (20%) and 7 (3.5%) samples tested positive for *E. coli* and *E. coli* O157: H7, respectively. Cheese (40%), raw milk (32%), yoghurt (25.71%) and beef (13.84%) were the foods with higher recovery rates. The majority of *E. coli* O 157: H7 isolates came from raw milk (12%), followed by cheese (5.71%) and beef (3.17%), however, pasteurized milk and yoghurt did not contain the pathogen.

Toro *et al.* (2018) aimed to isolate and characterize STEC non O157 from 430 ground beef samples from retail shops in the city of Santiago, Chile and obtained 56 isolates, 55 (98.2%) of them fermented sorbitol and six (10.7%) were resistant to Tellurite.

Rahman *et al.* (2020) collected 600 chicken meat swab samples, 75 each, of broiler and layer chickens, from four districts of, Sylhet, Moulavibazar, Sunamganj and Habiganj and obtained 381 *E. coli* isolates (63.5%) (197 from broiler and 184 from layer chicken).

Bhardwaj *et al.* (2021) examined 60 samples of dairy and meat products, in which 60% meat samples were positive for *E. coli*.

Dasgupta and Kumar (2021) collected 80 food samples (39 raw fruits, 25 raw milk and 16 raw meat) from

Silchar to perform analysis of the molecular epidemiology of *E. coli* using PCR. The prevalence of *E. coli* was found to be 10.25% in fruits, 12% in milk and 18.75% in meat samples.

Gutema *et al.* (2021) examined 127 beef samples in the Ethiopian town of Bishoftu and isolated 6.3% *E. coli* O157 from 127 beef samples.

Debbarma *et al.* (2022) studied 180 samples (90 each, of beef and chicken meat) in Mizoram and recorded that 83.33% beef samples and 80.00% chicken contained *E. coli*.

Hessain *et al.* (2015) assessed the prevalence and molecular characteristics of *E. coli* O157:H7 from 370 meat samples (200 raw meat and 170 meat products) from abattoirs and markets in Riyadh, Saudi Arabia and obtained 11 (2.97%) isolates of *E. coli* O157:H7.

Kumar *et al.* (2022) processed 100 samples, 50 each from goat and sheep and yielded 40 *E. coli* isolates (24 from goat and 16 from sheep), which were identified biochemically and confirmed by PCR.

Rahman and Ahmed (2022) conducted a study to isolate *E. coli* from 40 food samples comprising 20 each of, frozen milk and chicken meat from retail markets in Barishal city and recorded 20 chicken meat samples 15(75%) to be positive for *E. coli*.

Swetha *et al.* (2022) designed a study to identify Shiga toxin producing *E. coli* by the presence of virulence markers and PCR characterization of isolates from meat samples. A total of 150 meat samples comprising 50 of each beef, chicken and mutton were procured from retail shops in Chennai and subjected to conventional and molecular techniques. Out of 150 samples, 71 presumptive *E. coli* isolates recovered by conventional method and 61 isolates were confirmed by PCR.

Othman *et al.* (2023) conducted a study to isolate *E. coli* O157:H7 and detect *uidA*, *Stx1* and *Stx2* genes from 504 samples of meat and worker's hands from diverse areas in Mosul city. Total 138 isolates were recovered with the high prevalence depicted in the samples of worker's hands 4 (20%).

Public health significance

E. coli O157:H7 is mainly pathogenic to humans though bovine reservoirs (Caprioli *et al.*, 2005; Pennington, 2010; Soderlund *et al.*, 2012). It causes rarely diarrhea in cattle and other animals (Brown *et al.*, 1997) due to the difference in distribution of Gb3 receptors between cattle and humans (Smith *et al.*, 2002). *E. coli* O157:H7 infection is transmitted by faecal-oral route through contaminated food or water. The incubation period is 3 to 4 days, but may vary in the range of one to ten days (CDC, 2005).

The VTEC can cause a variety of disorders, particularly in children, the elderly and immuno-suppressed individuals, varying from mild diarrhea to HC, HUS and TTP (Gage *et al.*, 2001). Bloody diarrhea, abdominal cramps and little to no temperature are some of the clinical signs and symptoms of infection. The sickness passes in 5-10 days. The faulty diagnosis of appendicitis or

intussusception due to a STEC infection may lead to unnecessary surgical treatments (Griffin, 1995).

Hemorrhagic colitis

In 1982, the Centre for Disease Control (CDC) looked into two Hemorrhagic Colitis (HC) outbreaks in Michigan and Oregon. They found 47 cases with symptoms of severe stomach pain and profuse bloody diarrhea but no signs of infection by known enteric pathogens. In individuals with STEC infection, Pavia *et al.* (1990) used a barium enema or colonoscopy to observe the right-side intestinal inflammation. According to Griffin and Tauxe (1991), HC is characterized by excruciating cramping in the abdomen, bloody faeces, little to no fever and colonic mucosal oedema. Peritonitis, sub ileitis and perforation were reported as intestinal problems that ultimately required surgery. Cognitive impairment, aphasia, epileptic seizures, oculomotor abnormalities, myoclonus and headache were among the neurological consequences, although there was no morphologic evidence of microbleeds, thrombotic vascular occlusion, or ischemia infarction. One to four percent of patients with symptomatic HC brought on by EHEC infection pass away from complications, mostly the elderly or those with coexisting chronic illnesses.

Hemolytic uremic syndrome

G.I. tract infections brought on by STEC infection are typically linked to Hemolytic Uremic Syndrome (HUS). Two-thirds of patients in the acute stage of the illness need renal replacement medication and the majority of those individuals restored renal function. HUS is the frequent cause of acute kidney injury in young children. It was firstly identified by Gasser *et al.* (1955) as a separate clinical entity characterized by acute renal failure, thrombocytopenia and microangiopathic hemolytic anaemia. Renal failure, severe hypertension, myocarditis and/or neurological disorders are chief causes of death in the acute phase (Robson *et al.*, 1993). Acute renal failure, edema and hemolysis that cause thrombocytopenia, increased creatinine and lactate dehydrogenase are the hallmarks of HUS. When some individuals genuinely don't recover from bloody diarrhea, clinical deterioration or the development of HUS happens rapidly, frequently within 24-36 hours. According to Pickering *et al.* (1994), among HUS survivors, 10% experienced chronic renal failure and 40% had renal insufficiency or other lingering effects. Following STEC exposure, 38 to 61% of people got HC, 3 to 9% expressed sporadic infections and 20% showed epidemic forms that proceed to HUS (Banatvala *et al.*, 2001; Mead and Griffin, 1998). Scheiring *et al.* (2008) observed that 3-5% of children with STEC HUS die from their infection whereas 10-15% developed HUS.

Thrombocytic thrombocytopenic purpura

This illness is thought to be an elderly expression of HUS, with neurological involvement being more severe and a mortality rate as high as 50% (Griffin, 1995).

There is presence of *E. coli* and toxigenic genes which are of public health importance, it is therefore necessary to educate people regarding the dangers associated with the improper handling and contamination of meat. *E. coli* is frequently employed as a marker for fecal contamination, because of ubiquitous nature this organism in the tropics the association is questionable. Improper handling procedures may encourage the spread of pathogen. In order to limit the occurrence of these food-borne diseases to a minimal, appropriate surveillance techniques are required.

CONCLUSION

E. coli is one of the major inhabitants of the intestinal tract of animals, including human being and emergence of this bacterium as a food borne pathogen has a significant impact on the food quality. The pathogenic strains like *E. coli* O157:H7, can cause a range of foodborne illnesses, varying from mild diarrhea to severe conditions like hemorrhagic colitis, hemolytic uremic syndrome and thrombotic thrombocytopenic purpura, which can lead to kidney failure and even death, especially in vulnerable populations like children and the elderly and immunocompromised persons. The sources of *E. coli* contamination include livestock, poor hygiene practices during meat processing, improper food handling and more. In addition to meat, *E. coli* can be found in milk and dairy products, posing added risks to public health. Prevention of *E. coli* contamination can reduce the associated foodborne illnesses. For the instance, public awareness and educating them, regarding safe food handling and cooking practices are crucial. Proper hygiene measures during meat processing and stringent food safety regulations are necessary to minimize the risk of *E. coli* contamination. Additionally, robust surveillance and monitoring techniques should be in place to ensure the safety of meat products and protect the public from potential health hazards. The rapid growth of the global population and the increasing demand for meat products make it essential to address the issue of *E. coli* contamination seriously. Failure to do so could lead to more foodborne outbreaks, straining healthcare systems and posing a significant threat to public health. It is imperative that the food industry, regulatory authorities and consumers should work together to ensure the safety of meat and dairy products and reduce *E. coli* related illnesses in the end users of animal origin foods.

Conflict of interest

All the three authors have no conflict of interest to declare.

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