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REVIEW

The Importance of *Escherichia coli* O157:H7 as Foodborne Pathogen

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식품 병원균인 *Escherichia coli* O157:H7의 중요성

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In the last two decades *Escherichia coli* O157:H7 has been identified as an important foodborne pathogen. As a result of a foodborne outbreak at 1982, *E. coli* 0157:H7 was first recognized as an important human pathogen of public health concern, becoming part of the list of possible causative agents of bloody diarrhea. This incident opened the doors to a better understanding of illnesses and specific characteristics associated with this pathogen. Infection with this foodborne gram negative bacterium can lead to an illness designated as hemorrhagic colitis, possibly complicating further into hemolytic uremic syndrome (HUS) and/or thrombotic thrombocytopenic purpura (TTP). *Escherichia coli* O157:H7 is not the only pathogen associated with HUS. Other human pathogens such as *Salmonella; Shigella, Yersinia, Cmnpylobacter* and enteroviruses have also been linked to this syndrome (Fong *et al.*, 1982). Consumption of under-cooked contaminated ground beef is considered as the cause of the majority of outbreaks and illnesses caused by *E. coli* O157:H7 (Wells *et al.*, 1983). The Centers for Disease Control (CDC) and Prevention indicated in 1991 that the majority of *E. coli* O157:H7 outbreaks have been the result of transmission of this microorganism via foods of the bovine origin (Hawkins and Orme, 1995). Borczyk *et al.* (1987) and Chapman *et al.* (1993) stated that cattle are found consistently to be a reservoir of this microorganism in the environment. Because of this speculation, most of the scientific work and industry efforts are concentrated in monitoring this pathogen in beefer cattle. However *E. coli* 0157:H7 is not frequently found in cattle or cattle products. After analyzing over 3000 samples in 1996, the Food Safety Inspection Service (FSIS) reported a 0.06% prevalence rate for *E. coli* O157:H7 in ground beef indicating a very low level of natural occurrence of this pathogen (NFPA, 1996). The contamination of beef with this pathogen is believed to occur during slaughter, dressing, handling and storage of the animal products since the internal tissues of healthy animals are considered sterile (Nortje *et al.*, 1990). Even though *E. coli* O157:H7 is present in the meat supply in low numbers, the pathogenicity of this bacterium at low microbial numbers is of significant public health concern. Because this pathogen is endemic to cattle and can cause illnesses through contaminated beef, the United States Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS) declared *E. coli* O157:H7 as an "official adulterant" (FDA, 1996). This declaration implies and requires the exclusion of this pathogenic bacterium fiom meat products. In order to ensure food safety, it is imperative for the food industry to routinely monitor for the presence of this pathogen. In order to improve the quality of food products it is also necessary to establish systematic approaches like the implementation of Hazard Analysis Critical Control Points (HACCP) for identification and control of foodborne pathogenic microorganisms (Kodikara *et al.*, 1991.) The importance of *E. coli* O157:H7 in food safety became so significant that in 1992 this pathogen became the third most costly foodborne pathogen in the U.S. in terms of medical costs and loss of productivity (Durham *et al.*, 1996) and in 1993 it was established as the "Microorganism of the Year". The scientific community and governmental organizations

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are putting their efforts together to create better monitoring programs and techniques to improve and ensure that food is free from this pathogen. In June 1993, the Association of State and Territorial Public Health Laboratory Directors, together with the Council of State and Territorial Epidemiologists recommended that all clinical laboratories screen or monitor *E. coli* O157:H7 with Sorbitol MacConkey Agar from at least all bloody stool specimens (Council of State and Territorial Epidemiologists, 1993). Also, in order to keep track of the presence of this bacterium, the USDA-FSIS has been routinely monitoring this pathogen from the ground beef supply since 1994 (Hawkins and Orme, 1995). Currently, the CDC recommends testing for *E. coli* O157:H7 as a possible diarrhea causing microorganism in all clinical laboratories. Boyce *et al.* (1995) suveyed 129 laboratories performing stool cultures and found that only 70 (54%) reported screening either all stools or all Moody stools for *E. coli* O157:H7. Beef is not the only food product currently associated with hemorrhagic colitis, HUS or TTP. Other food products such as raw milk, apple cider, drinking water, and other ground meats have also been linked to these diseases. Recently cantaloupe and mayonnaise were also implicated as the cause of hemorrhagic colitis transmitted by *E. coli* O157:H7. When *E. coli* O157:H7 was first classified as a harmful enteric foodborne pathogen, its isolation and identification procedures involved screening sorbitol-negative isolates fiom MacConkey Sorbitol Agar (MSA) in combination with a specific antiserum using a tube agglutination assay. Today it has been shown that the *E. coil* strain O157:H7 is not the only sorbitol negative *E. coli* strain. The complete identification or monitoring of *E. coli* O157:H7 requires the proper isolation of the pathogen and confirmation of the culture by both the O157 and the H7 flagellar antigens. Testing for the H7 antigen is routinely accomplished by using an H 7-specific antiserum and an agglutination assay. Traditional or conventional methods utilized in the monitoring (isolation, detection or enumeration) of *E. coli* O157:H7 from other microorganisms or other *E. coli* strains from raw or cooked foods usually involve timeconsuming cultivation procedures. These procedures require enrichment of the sample in a selective medium for at least 24 hours. Enrichment of the sample is usually followed by an isolation procedure and further serological or biochemical confirmation tests. Confirmation assays can take an additional 24 to 48 hours to obtain accurate and definitive results. The overall identification procedure, including serotype confirmation, can take from 48 to 72 hours from the time the test started. Sometimes cultural methods of isolating this pathogen from food samples require 6 to 7 days to make a positive confirmation (Okrend and Rose, 1989). Today a variety of methods for the immunological detection and biochemical identification of *E. coli* O157:H7 have been described (Kleanthous *et al.*, 1988; Padhye and Doyle, 1991; Smith and Scotland, 1988; Todd *et al.*, 1988; Tyler *et al.*, 1991), resulting in the improvement of techniques for the monitoring of this pathogen. Current rapid techniques for monitoring *E. coli* O157:H7 are mostly concentrated in the screening, detecting or characterizing of this pathogen. More methods for determining the degree of contamination of the food product (quantitative or enumeration methods) are needed to differentiate this pathogen from other verotoxigenic *E. coil* (VTEC) producing strains. Recently, fast and accurate techniques for the early monitoring of this foodborne pathogen have been developed and tested. Because this pathogen can cause infection in low concentrations (1 to 10 cells), Jinneman *et al.* (1995) reported that any newly developed rapid or improved technique should effectively be capable of screening or identifying *E. coli* O157:H7 at low numbers. Johnson *et al.* (1995) indicated that lots of ground beef containing 0.9 to 4. 3 Colony Forming Units per gram (CFU/g) of this pathogen had been implicated in the 1993 outbreak in the northwestern United States. The presence of this pathogen at low initial numbers, in the presence of high levels of competing microbial flora presents a difficult task for medical and food microbiologists. Past and current incidences of illnesses and outbreaks associated with *E. coli* O157:H7 indicate that there is still a lot to be learned about the pathogen such as route of illness transmission, sources of contamination, related toxins and ability to cause human infections through different foods. The field of rapid methods and automation in microbiology involves improved techniques and procedures to rapidly isolate, detect, enumerate and characterize microorganisms including *E. coil* O157:H7. This study addresses specific rapid and improved techniques for monitoring this pathogen together with the most significant criteria used in the selection of an appropriate *E. coli* O157:H7 monitoring technique.

History of Pathogenic *Escherichia coli* Groups

The name *Escherichia coli* was given to this particular species of bacterium in honor of its discoverer T. Escherich. *E. coli* is a gram negative, facultative anaerobic microorganism commonly found in the intestine of animals and humans. Since this bacterial species is normally found in the intestine, this and other coliforms are used as indicators of fecal contamination of foods and potable water. The majority of *E. coli* strains are non-pathogenic but there are some strains that are able to cause illnesses in humans. These pathogenic strains of *E. coli* are directly responsible for causing diarrhea when ingested from contaminated food sources. Pathogenic *E. coli* are divided into five major groups. Each of them displays distinct symptoms of infection, serogroups and pathogenicity. The pathogenicity and epidemiological effect of these *E. coli* strains have been described by Levine (1987). Included in these strains are the enteropathogenic *E. coli* (EPEC), isolated first in the 1950's. These pathogenic *E. coli* causes human diarrhea by the production of enterotoxins. The second type of pathogenic *E. coli* includes the enterotoxigenic *E. coli* (ETEC). These strains were identified in the 1970's and its different serogroups can produce either a heat labile (SL), a heat stable (ST) enterotoxim or both (Notermans *et al.*, 1991). The enteroinvasive *E. coli* (EIEC), also isolated in the 1970's are diarrhea causing microbial strains that invade the intestinal epithelium, sometimes producing severe dysentery-like symptoms. Enteroaggregative *E. coli* (EaggEC) is a group of the most recent *E. coli* strains found to produce illnesses in humans. The last pathogenic *E. coli* group is considered the most common of the five. This group is denoted as enterohemorrhagic *E. coli* (EHEC), also known as "verotoxigenic *E. coli*" or VTEC. The EHEC group was first identified in 1977 but it was not until 1982 that this group became very well known with the discovery of its most important serogroup, *Escherichia coli* O157:H7. A previous isolation of this serotype in calves with colibacillosis was made in Argentina in the 1970's, (Bettelhein, 1996) but this serotype was not identified in the United States until 1982. This VTEC was first isolated when a group of people became ill due to the consumption of contaminated ground beef from a fast food restaurant chain in 1993. *E. coli* O157:H7 is believed to be directly associated and responsible for causing hemorrhagic colitis, hemolytic uremic syndrome and thrombotic thrombocytopenic purpura, and since its first reported outbreak, this pathogenic bacterium has been increasingly identified as the causative agent of food related illnesses in humans and a threat to public health.

Description of *Escherichia coli* O157:H7

Escherichia coli O157:H7 is gram negative flagellated bacilli that belongs to the coliform and the *Enferobacteriaceae* family, a group of bacteria normally found in the intestine and feces of animals and humans, This pathogen is classified as an enterohemorrhagic *E. coli* (EHEC) due to its ability to cause hemorrhagic colitis (severe bleeding of the colon), HUS and TTP in humans. The pathogenic properties of this particular *E. coli* serotype are believed to be a consequence of the production of two different toxins. These verotoxins (VT), also known as Shiga Like Toxins or SLT, have a cytophatic effect in Vero and HeLa cells and damage the kidney. *E. coli* O157:H7 cytotoxins or SLTs have biological properties similar to Shiga toxins produced by Shigella dysenteriae (Konowalchuck *et al.*, 1977; O'Brien and La Veck, 1983). The type of toxins produced by *E. coil* O157:H7 are easily inactivated by heat (heat labile). Even though this is the most common serotype or strain of *E. coli* associated with human illnesses, it is not the only verotoxin producing *Escherichia coli*. Other VTEC *E. coli* strains such as serotypes O111:H-, O111:H8, O113, O91 and O26:H11 have also caused serious human illnesses in the United States and in other parts of the world.

Toxins produced by *Escherichia coli* O157:H7

In 1955 when hemolytic uremic syndrome was first described, its pathogenicity was believed to come fiom a toxin produced by *Shigella dysenteriae* (Shiga Toxin type 1 or STI). *Escherichia coli* O157:H7 toxins, just like toxins produced by other *E. coli* strains, are believed to play a significant role in the development of *E. coli* related illnesses. Some enterohemorrhagic *E. coli* (EHEC) have the ability to produce a cytotoxin or verocytotoxin that has a cytopathogenic effect on growing Vero cells and the kidneys. These verotoxins are also believed to be directly associated with the production of hemorrhagic colitis, possibly causing

further acute renal failure (a product of HUS), TTP and even death in young children, elderly people and immunocompromised individuals. The mechanism of action of these toxins are not yet fully understood (Farmer *et al.*, 1988). The type of toxin produced by *E. coli* O157:H7 can be easily denatured with heat (heat labile). The importance of these toxins in verotoxigenic *E. coli* (VTEC) strains was not discovered until 1983. Since their discovery, these toxins have been directly associated with the pathogenicity of hemorrhagic colitis and HUS. Verotoxin producing *E. coli* strains can produce either one or both types of verocytotoxins. These toxins are classified as verotoxin 1 and 2, (VTI and VT2) (O'Brien and Holmes, 1987). VT2 has been further classified into different subgroups like VT2C. Both of these toxins are also referred as Shiga-Like Toxins 1 and 2 (SLT1 and SLT2), due to their genetic and antigenic similarity to toxins produced by *Shigella dysenteriae*. The genus *Shigella* and *Escherichia* are so closely related to the point that both genera share the same antigens. *Shigella dysenteriae* type 1 has the same "O" antigen as *Escherichia coli* O1 and could be given the structure O1:H-" (Bettelheim, 1996). VTI, also know as SLTI, is antigenically related to Shiga Toxins while VT2 (SLT2) is antigenically different from Shiga Like toxins. Both toxins are believed to have the same effect on Vero cells. Other *E. coli* strains can also produce SLT1. Even though SLT are highly associated with the production of hemorrhagic colitis, HUS and TTP, it does not appear to be any evidence to support that illnesses caused by *E. coli* serotype O157:H7 are a direct cause of the production of SLT. Isolation of this serotype from diarrheal stools, especially with blood is indicative of a Shiga Like Toxin-producing strain (Karmali, 1989). Some of the current techniques used for the identification of *E. coli* O157:H7 toxins include Oxoid's Latex test (Oxoid, 1996), DNA hybridization (to detect VT genes), Vero cell assays, the MERIDIAN EHEC-Tek (ELISA) and Western blot.

Incidence and Epidemiology

Before 1982, not much was known about the foodborne pathogen *Escherichia coli* O157:H7. In that year this pathogenic microorganism was first recognized as the causative agent of specific human illness, with outbreaks in Oregon and Michigan (Bean and Griffin, 1990). Not too long after this outbreak, this pathogenic *E. coli* strain was shown to cause diarrhea in laboratory animals such as infant rabbits (Farmer *et al.*, 1983) and gnotobiotic piglets (Frances *et al.*, 1986). The Oregon and Michigan incidences were directly related to the consumption of undercooked ground beef in different fast food restaurants. That same year another outbreak was reported in a nursing home in Ontario, Canada, also associated with the consumption of contaminated ground beef. In 1986 a couple of outbreaks were reported; one in a nursing home in Alberta, Canada, (a case directly associated to the consumption of ground beef) and another in Wisconsin from a product of the ingestion of contaminated raw milk. One year later in 1987, two new outbreaks emerged; one in Utah and another in kindergarten children in Ontario, Canada. These two cases were associated with the consumption of undercooked hamburger and raw milk, respectively. In 1988 some cases of *E. coli* O157:H7 illnesses were linked to the consumption of raw beef in a junior high school in Minnesota. After this, more isolated incidences occurred in the following years. The most prominent cases of illnesses caused by *E. coli* O157: H7 occurred in late 1992, early 1993 in Washington, Idaho, Nevada and California (Scotland *et al.*, 1991). As a result of these incidences many individuals became ill and small children died (Tsai *et al.*, 1996). All of those cases were associated with the consumption of inadequately cooked ground beef at multiple outlets of a fast food chain (American Gastroenterological Association, 1995; Barrett *et al.*, 1994; Bell *et al.*, 1994). In these incidences more than 700 people became ill, 195 were hospitalized and 4 died (American Gastroenterological Association, 1995). The most recent incident of *E. coli* O157:H7 infection occurred in late October of 1996. In this outbreak over 70 people became infected as a result of the consumption of a specific brand apple cider. As a consequence of this outbreak, 66 people became ill. and 1 toddler died. All these incidences and outbreaks have attracted the attention of governmental organizations, health institutions, the scientific community, the microbiology field and especially the meat industry. As a result, the meat industry and official health organizations have been creating better, more efficient inspection and monitoring programs to control or eradicate *E. coli* O157:H 7 from the meat supply and from other food groups. Also more emphasis have been given to the education of con-

sumers about the proper handling and preparation of foods in order to help control, reduce and prevent further *E. coli* O157:H7 incidences and outbreaks.

Foods associated with *Escherichia coli* O157:H7 — When the first cases of illnesses and outbreaks of *Escherichia coli* O157:H7 occurred, consumption of contaminated ground beef was believed to be the pathogen's only method of disease transmission. Today, cattle is considered to be a natural reservoir of this pathogen in the environment. Martin *et al.* (1986) and Borczyik *et al.* (1987) reported that *E. coli* O157:H7 has been isolated from fecal samples of calves and heifers. Dairy cattle has also been shown to be a reservoir of this pathogen (Borczyik *et al.*, 1987). Ingestion of contaminated ground beef is considered to be the source of infection in most *E. coli* O157:H7 outbreaks reported (Belongia *et al.*, 1991; Ostroff *et al.*, 1990; Pai *et al.*, 1984; Ryan *et al.*, 1986.) Even though beef has proven to be the pathogen's main vehicle of illness transmission, other food groups have also been linked to *E. coli* O157:H7 illnesses. Some of these other foods include drinking water (McGrowan *et al.*, 1989; Scotland *et al.*, 1991; Swerdlow *et al.*, 1992), ham, cheese, turkey rolls, raw milk(Karmali, 1989), cold sandwiches and vegetables (Griftin and Tauxe, 1991), radish sprouts, new potatoes, pork, poultry, lamb and other ground meats (Doyle and Schoeni, 1987). A study by Doyle and Schoeni in 1987 reported incidences of *E. coli* O157:H7 in 3.7% of the retail beef, 2.0% of the retail lamb and 1.5% of the retail pork and poultry. Cantaloupe and mayonnaise have also been recently suspected to be vehicles of hemorrhagic colitis transmission (Anonymous, 1993). Recently, two outbreaks were linked to the consumption of low-pH foods, a food group usually considered free from *E. coli* O157:H7; an outbreak in Massachusetts was associated with drinking one brand of unpasteurized apple cider (Besser *et al.*, 1993) and the other outbreak was linked to the ingestion of food containing mayonnaise from an Oregon restaurant chain (Keene *et al.*, 1993). Laboratory experiments conducted with both of these food products showed that even though *E. coli* O157:H7 was dying rapidly in these acid foods at room temperature, the pathogen survived for several weeks at refrigeration temperatures (Besser *et al.*, 1993; Weagant *et al.*, 1994). Sewage contaminated drinking water has also been implicated in the transmission of *E. coli* O157:H7 illnesses.

Illnesses associated with *Escherichia coli* O157:H7 — Due to the ability of *Escherichia coli* O157:H7 to cause illness with a low inoculum, public education and early pathogen detection are considered to be the most important factors involved in the prevention and proper management of outbreaks and diseases caused by this pathogenic microorganism. *E. coli* O157:H7 is believed to cause enteric related illnesses in humans as frequently as *Salmonella* and in some cases it can surpass *Shigella* illnesses. In studies comparing the isolation rate of *E. coli* O157:H7 with those of other bacterial enteric pathogens, *E. coli* O157:H7 has commonly been isolated more frequently than *Shigella* species (Bokete *et al.*, 1993; Cahoon and Thompson, 1987; Gransden *et al.*, 1986; MacDonald *et al.*, 1988; Marshall *et al.*, 1990; Pai *et al.*, 1988). Preliminary data from a U.S. study showed that *E. coli* O157:H7 was isolated from stool specimens with visible blood more frequently than any other pathogen (Ries *et al.*, 1993). The initial infection with this pathogen can be acquired by fecal contamination of foods and water, then the illness can be further transmitted via person to person contact. Infection with this pathogen may cause a wide variety of illnesses ranging from mild, non-bloody diarrhea to extreme hemorrhagic colitis. The initial symptoms of infection include watery diarrhea and abdominal pains that could last a couple of days. The rapid progression or complications of infection can lead to other severe diseases such as hemolytic uremic syndrome or HUS, (Karmali *et al.*, 1985; O'Brien and Holmes, 1987), the leading cause of acute renal failure associated verocytotoxins, microangiophatic hemolytic anemia and thrombotic thrombocytopenic purpura, (Karmali *et al.*, 1985; O'Brien and Holmes, 1987) a disease that is similar to HUS, but also can cause human central nervous system damage. Symptoms associated with HUS include severe abdominal cramping, possible bloody diarrhea and the presence or absence of fever (Riley *et at.*, 1983). These symptoms can occur from a couple of days to a week after infection. At this time copious amount of blood (evidence of colonic inflammation) can be found in the patient's stool. Even though infection with *E. coli* O157:H7 does not always produce a bloody stool, it is frequently associated with HUS. Neill *et al.* (1987) isolated *E. coli* O157:H7 from seven or 58% of 12 patients having bloody diarrhea preceding the formation of HUS. Complications like HUS can severely af-

fect and damage the kidneys and kidney cells. Also severe complications can result in the deaths of young children and/or immuno-compromised individuals (Griffin *et al.*, 1988 Karmali, 1989; Kovacs *et al.*, 1990; O'Brien *et al.*, 1993; Ostroff *et at.*, 1990; Thompson *et al.*, 1990). Infection with *E. coli* O157:H7 has a mortality rate of 10% (Oxoid/Unipath. Ogdensburg, New York). The mechanism in which this pathogen causes illness is not yet well understood, but its pathogenicity is believed to be directly associated with the production of cytotoxins 1 and 2, (VT1 and VT2), The degree of pathogenicity of HUS and TTP could very much depend on the ability of *E. coli* O157:H7 to produce Shiga Like toxins (Griffin *et al.*, 1988; Kannali *et al.*, 1985). Other *E. coli* serotypes can also produce verocytotoxin, but they do not seem to be normally associated with bloody diarrhea. Griffin and Tauxe (1991) stated that although other *E. coli* serotypes have been proven to produce one or both of these cytotoxins, cases in which Shiga-Like toxin producing *E. coli* (different from *E. coli* O157:H 7) cause bloody diarrheal disease are uncommon. The clinical diagnosis of human infections with this pathogen can be achieved by isolating the bacterium from the patient's stool.

Culturing Characteristics Unique to *Escherichia coli* O157:H7

One of the most distinctive characteristics that differentiates *Escherichia coli* O157:H7 from other enterics is its inability to ferment D-sorbitol in a 24 hour period, (Kleanthous *et at.*, 1988 March and Ratnam, 1986) or that it ferments this compound very slowly, when compared to most other *E. coli* strains (Wells *et al.*, 1983; Fasmer and Davis, 1985). This characteristic applies only partially to the monitoring of this pathogen, since most verotoxigenic *E. coli* O157 strains do not seem to ferment sorbitol. Also, as many as 20% of all *E. coli* strains may be sorbitol negative (Biolog Inc. Hayward CA). Another charactetistic relatively specific to *E. coli* O157:H7 is the pathogen's production of white or colorless colonies on MacConkey Sorbitol Agar (MSA), when used as a primary medium. Although this is true in most occasions, there is evidence to support that other species (at least 45 species) of enteric bacteria can also grow as colorless colonies on MSA (Biolog Inc. Hayward CA). Other enterics, or *E. coli* strains that ferment sorbitol produce pink to red color colonies on this medium. One characteristic that seems to be very specific to *E. coli* O157:H7 is the fact that this pathogen lacks the production of β-glucuronidase, thus gives a negative reaction in the methyllumbelliferyl-β-glucuronide (MUG) fluorogenic assay. Other *Escherichia* species like *Escherichia henrzannii* are sorbitol negative and can also form an agglutination under O157 antiserum. Although both bacterial species have these reactions in common, *E. hemtmulii* can be differentiated from *E. coli* species by its ability to ferment cellobiose (March and Ratnam, 1989). Even though some of the culturing characteristics used to isolate or detect *E. coli* O157:H7 can in very few occasions cross react with other microorganisms or other *E. coli* strains, the general monitoring of this pathogen it is still based on the screening of sorbitol negative and MUG negative results.

Growth and Survival Characteristics of *Eshcerichia coli* O157:H7

Specific characteristics associated with the growth and survival of *E. coli* O157:H7 play a key role in the technologies used to isolate, identify or enumerate this pathogen. Table 1 lists some of these characteristics (Tsai *et al.*, 1996).

Table 1. Growth and survival characteristics associated *Escherichia coli* O157:H7

Temperature	• Minimum growth temperature: 6.5°C to 6.8°C • Maximum growth temperature: 37°C to 45°C depending on the strain. • Grows rapidly in TSB between 30°C to 42°C • Grows poorly at 44°C to 45°C • *E. coli* O157:H7 is more heat sensitive than *Samlonella.* (D- value of 9.6 at 64.3°C/14°F) • Survives temperatures of 54°C for 40 minutes without significant loss of viability (Oxoid/ Unipath Ogdensburg N.Y.). • Survives freezing (in ground beef) at -20°C for 9 months.
pH	• Acid tolerant in low pH foods (survives up to 31 days at a pH of 3.6 in apple cider).
Atmospheric Modifications	• Inhibited only at CO_2 concentrations higher than 60%

Other Verotoxigenic *Escherichia coli* (VIEC) Serotypes

Before *Escherichia coli* O157:H7 was isolated in 1982 many severe diarrhea cases were attributed to *Shigella* and *Salmonella* toxins. Today it is well known that these two microorganisms are not the only pathogenic bacteria to cause these human illnesses. *E. coli* O157:H7 is not the only verotoxigenic or enterohemorrhagic *E. coli* serotype that can produce Shiga-Like toxins. Other non- O157:H7 or O157:H- serotypes can also produce these toxins (VT1 and VT2) causing hemorrhagic colitis, hemolytic uremic syndrome or other toxin related illnesses in humans. Table 2 shows a list of different non-*E. coli* O157:H7 or O157:H-, EHEC serotypes believed to be associated with human illnesses and that are now emerging as possible frequent human pathogens.

Since the first Shiga-Like toxin-producing strains of *E. coli* were isolated in 1963 many other types of verotoxigenic *E. coli* serotypes have emerged. Serotypes such as O26:H11, O126:H21, O39:H18, O15:H2, O128:H2, O103:H, O68:H-, O26:H- and O- :H111 have been isolated from humans suffering some sort of gastrointestinal illness. Some other cases of human illnesses have also been reported, linking serotypes O26:H-, O111:H-, O111:H8, O104:H21 and O 113:H21 to gastrointestinal illnesses in and out of the United States. Today many more verotoxin or cytotoxin producing *E. coli* serotypes have been added to this list. Outbreaks of these serotypes clearly indicate that *E. coli* O157:H7 is not the only pathogenic *E. coli*, causing hemorrhagic colitis, HUS or TTP in humans. A study in Ontario Canada in which 1,131 dairy cows and 659 calves were monitored for SLT producing *Escherichia coli* showed that 60% of the cows and 100% of the calves had a serotype other than O 157:H7 (Bettelheim, 1996). From all the non-O157:H7 EHEC serotypes mentioned, there are some that are believed to appear at a higher frequency in humans causing serious gastrointestinal illnesses. Serotypes O26, O111, O113 and O 91 have been considered enteropathogenic, occasionally causing human illnesses and outbreaks world wide. Different *E. coli* O26 serotypes like nonmotile O26:H- and motile O 26:H11 are believed to produce SLTI, frequently leading to hemorrhagic colitis and HUS in humans. Non-motile and motile strains of serotype O111 such as O111:H- and O111: H8 together with strains of serotypes O113 (O113:H21 and O113:H4) and O91 strains (O91:H- , O91:H14 and O91:H 21) have also been linked to these hemorrhagic illnesses. A specific *E. coli* serotype is not necessarily indicative of pathogenicity. Some other SLT producing serotypes like O 153:HI25, O5:H-, O163:H19 and O45:H2, associated with hemorrhagic colitis and HUS around the world have also been isolated from perfectly healthy animals (Bettelhein, 1996). These cases indicate that for some serotypes,there is not an effective way to determine if they are pathogenic to humans or not. As time passes, more and more pathogenic *E. coli* serotypes probably will emerge and will be added to the potential pathogen list. Today some of the commercially available techniques for the identification of pathogenic *E. coli* serotypes involve agglutination tests especially designed to identify the *E. coli* "O" antigen like O157, O26 and O111 only (Bettelheim, 1996). Serotype O111 has biochemical characteristics different from those of serotype O157; *E. coli* O111 is oxidase, sorbitol P-glucuronidase and MUG positive. Due to similarities in test reactions between this and other *E. coli* serotypes, more selective techniques with higher degrees of specificity are needed. Also, it is imperative for the scientific community, and the clinical and food microbiology industry, to be aware of these other *E. coli* serotypes. The realization of the importance of these serotypes will contribute to a better control of potential pathogens found in the food supply.

Table 2. Non-O157:H7 or O157:H- *E. coli* serotypes associated with human illnesses

O26:H11	O111:H-	O15:H2	O113:4
O126:H21	O111:H2	O91:H-	O113:H21
O128:H-	O111:H8	O91:H14	O103:H-
O128:H2	O104:H-	O91:H21	O68:H-

Adapted from Bettelheim, 1996

Detection Methods

Several methods have been used to detect *E. coli* O157: H7 and its toxin such as; conventional methods, like the MacConkey sorbitol agar; and rapid methods which include Petrilm™, randioimunoassay (RIA), Enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR).

MacConkey Sorbitol Agar (MSA)

MSA has been used as conventional methods in many countries to detect *E. coli* O157: H7. Since *E. coli* O157: H7. does not ferment sorbitol it produces of pink or colorless colonies on MSA. Serology agglutination tests can be used to

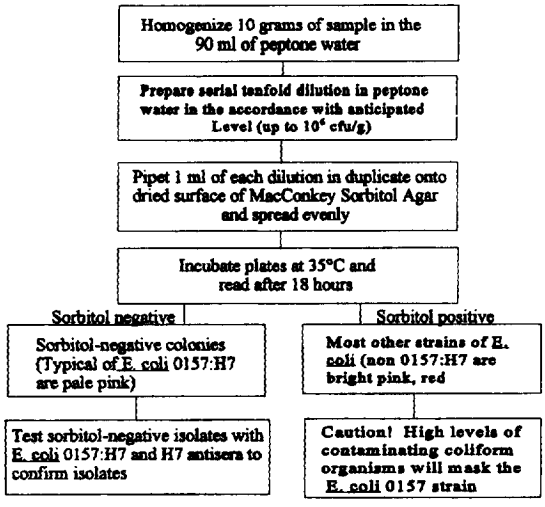

Fig. 1. Media and methods for isolation and identification of *Escherichia coli* O157:H7 in food.

confirm the results (March and Ratnam. 1989). Agglutination means the *E. coli* O157: H7. is present in the sample. The Fig. 1 shows the procedures for identifying *E. coli* O157: H7.

Radiomimmunoassay (RIA)

RIA kits contain the antibody which is combined with the substance (specimen). As the specimen reacts with or binds to an antibody, radioactivity will be generated and measured by a detector (Fung, 1996). The method is simple, but use is limited due to cost, requires more practice handling and disposing the radioactive material, and it takes a long time 24 h to get results. RIA has been used to test in more critical issues such as hormones, selected therapeutics drugs, or in most research.

Enzyme-linked immunosorbent assay (ELISA)

ELISA kits are similar to the RIA, with the exception that the enzyme will bind with the specimen. The procedure consists of preparing samples by mixing the target analyte, then the enzyme linked will react with the target analyte, follow by adding a reagent which will change the color. The color will be measured either qualitative or quantitative (Tsai, 1996).

Petrifilm™

After preparing the sample, 1 m*l* from the dilution is transferred to the center of the Petrifilm™, then sample is spread gently downward and pressured onto the center by the plastic spreader (Okrend, *et al.*, 1990). The Petrifilm™ should be incubated at 32°C for 48h. The *E. coli* colonies will be blue with gas. Nevertheless, *E. coli* O157:H Shiga like toxin II 7 will be a grey spot (Calicchia *et al.*, 1994). According to Fung (19%), Petrifilm™ is used to for *E. coli* count and viable cell count enumeration. Its characteristics is accepted by AOAC, simple and easy to use, reduces the time and media to get results, easier to read, and good for small laboratories.

Polymerase chain reaction (PCR)

Polymerase Chain Reaction (PCR) procedure can be used to confirm suspect microbial colonies and to screen for pathogenic microorganisms after enrichment such as Salmonella and toxin producing strains of *E. coli* O157:H7 (Qualicon, 1996). This PCR technique is also available for the detection of *Listeria* and *L. monocytogenes*. The *BAX™ Pathogen Screening Sysrem* is designed to detect *E. coli* O 157:H7 in food samples and in environmental systems. In the process of detecting this pathogen, the system targets and reproduces multiple copies of a specific *E. coli* O157: H7 DNA fragment to a level that can be detected using gel electrophoresis, This technique differs from many detection systems by targeting a bacterial gene, rather than the pathogen's antigen, resulting in more clear-cut "yes-no" results. The technique also simplifies the screening process by combining the primers, nucleotide and polymerase enzymes into a single sample tablet, already packaged inside the contained PCR tubes.

Two primary methods will improve the quality and eliminate or reduce the risk new techniques in food manufacturers and educate the consumers. New techniques in food processing mainly, apply a good sanitation and personal hygiene program, using the rapid methods to detect and monitor the pathogens, or applying the HACCP program in the large food manufacturing. Educating consumers is also more useful in preventing the risk of infection. Education should emphasize sanitation in the kitchen, handling food, washing hands after everything such as sneezing, touching sort, raw food, baby diaper etc., heating food to appropriate temperature, and given the consumers background about the kinds of disease which are caused by pathogens.

국문요약

지난 수년간 *Escherichia coli* O157:H7에 의해 오염된 식품으로 인한 발병이 지속적으로 증가하고 있고 이 병원균의 억제 및 검출에 대한 필요성이 인정되고 있는 상태이다. Hemorrhagic colitis, hemolytic uremic snydrome 그리고 thrombotic thrombocytopenic purpura와 같은 증세가 *E. coli* O157:H7에 의해 발생된다. 최근에 발생한 *E. coli* O157:H7에 의한 발병은 식품위생과 안전성에 대한 관심을 불러 일으키면서, 이 병원균을 억제하는 방법들에 대한 연구와 정확하고 빠르게 이 병원성균을 검출하는 방법에 대한 연구가 활발히 진행되고 있다. 이러한 관점에서 이 병원균의 성장 특성, 종류, 그리고 독소에 대한 기본인식이 필요하다. 이 논문은 *E. coli* O 157:H7의 기본적인 특징, 배양 특성, 독성(verotoxin 1 그리고 2)의 중요성, 그리고 식품으로 인한 발병 경로 및 균의 검출방법에 대해 기술하고 있다.

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