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O157: H7 SEROTYPE OF *ESCHERICHIA COLI* AS AN IMPORTANT EMERGING ZOONOSIS

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ABSTRACT

E. coli is a Gram negative facultative anaerobic bacillus found in the family *Enterobacteriaceae*, is widely distributed as part of the essential intestinal flora that maintains the physiology of the healthy host. *E. coli* O157:H7 is one of the virulent strains of the pathogenic *E. coli*. It was emerged as a zoonotic problem in 1982 because of changes in agriculture and in food preparation. *E. coli* O157:H7 is resistant to acid, salts, and antibiotics. However, it is susceptible to heat. Low infectious dose, up to 10 organisms, is required to cause infection in humans. *E. coli* O157:H7 produces Shiga-like toxins (SLTs) and causes hemolytic-uremic syndrome (HUS), hemorrhagic colitis (HC), thrombotic thrombocytopenic purpura (TTP) type of diseases in humans. In animals it is most common in beef farms, but the clinical disease is less common. Cattle are the main reservoir of this zoonotic infection, and the disease is transmitted by fecal contamination of meat, uncooked beef, and unpasteurized milk and milk products. Diagnosis of *E. coli* O157:H7 infection involves culture, Biochemical tests, serological tests and polymerase chain reaction (PCR). Prevention of the disease is by proper hygienic and good management procedures in food processing starting from farm to the food processing environment.

KEYWORDS: *E. coli* O157:H7, Hemolytic-uremic syndrome, Hemorrhagic colitis, Shiga-like toxin.

INTRODUCTION

The organism, *E. coli* O157:H7 is a Gram negative, facultative anaerobic, rode shaped, sorbitol and glucuronidase negative bacterium that infects the intestinal tract and produces a toxin that affects other parts of the body.[1, 2] It is a world wide emerging and virulent zoonotic pathogen representing an important health disorder to the consumer. It is the cause of many human deaths and illnesses in different parts of the world through consecutive outbreaks and causes enormous economic loss.[3, 4] It has been speculated that the two major factors that have given rise to the increased prevalence of *E. coli* O157:H7 infection are changes in agriculture (beef cattle production) and in food production (such as hamburger). It may have the ability to generate a higher 'tail' population and this coupled with the low infective dose, up to 10 organisms[5] has allowed the organism to become a major pathogen.[1,3, 5]

E. coli O157:H7 have been linked in humans with HUS, HC, and TTP.[3] HUS and TTP are serious illnesses characterized by kidney failure and destruction of the RBC leading to anaemia, respectively. Young children and elderly are at greatest risk of developing HUS or TTP following infection.[2] The severities of the disease ranges from mild uncomplicated diarrhea to HC with sever abdominal pain, bloody diarrhea, and little or no fever.[2, 6]

Although cattle appear to be the main reservoir of *E. coli* O157:H7, infected persons, animals including sheep, goats, pigs and poultry also transmit the disease. [2, 3, 5, 7] The herd prevalence of infection with *E. coli* O157:H7 in North America and European cattle herds ranges from 3-8% and 0.5 – 1.0 % of animals with a higher prevalence up to 5% in weaned calves and heifers.[3] Some survey reported the organism in dairy and beef cattle at 0.28% and 0.71% respectively. Other studies found much higher level of prevalence such as 15.7% of cattle over a year period. Monthly prevalence ranges from 4.8- 36.8% and were higher in the spring and later summer. [3]

Transmission to humans is principally through the consumption of contaminated foods. [2, 3, 6] Human infections with *E. coli* O157:H7 has a world wide distribution. Serotype O157:H7 has been isolated in outbreaks in Canada, Great Britain, and the United States. It has also been isolated in Argentina, Belgium, the former Czechoslovakia, and China.[8] These isolates were obtained from fecal samples taken from sporadic cases of hemorrhagic diarrhea submitted to public health or hospital laboratories for examination.

Little is known about the *E. coli* O157:H7 in Ethiopia. One study from diarrheic human patients in hospitals showed absence of the organism in stool.[9] This is far from conclusive and more studies should be done to determine the significance of the bacteria in our country (Ethiopia). However as the transmission of the infection are usually from consumption of uncooked animal products and poor personal hygiene both of which are prevalent in our living style, the possibility of the disease being important in Ethiopia cannot be underestimated. Therefore, the objective of this paper is to review and compile available literature information on the characteristics of the bacteria, about its occurrence in man and animals, its method of transmission, diagnosis and control of the disease which will increase our understanding about the disease.

LITERATURES REVIEW

E. Coli and its serotypes

E. coli, a Gram negative facultative anaerobic bacillus found in the family enterobacteriaceae, is widely distributed as part of the essential intestinal flora that maintains the physiology of the healthy host.[10, 11, 12] It is for this reasons that *E. coli* is considered as a good indicator of fecal contamination. Although most strains of *E. coli* are not, regarded as pathogens, they can be opportunistic pathogens that cause infection in immunocompromised hosts. There are, however, pathogenic strains of *E. coli* that when ingested, cause gastrointestinal illness.[2,13,14]

More than 700 serotypes of *E. coli* have been identified. The different *E. coli* serotypes are distinguished by their "O" and "H" antigens on their bodies and flagella, respectively, and "K" capsular antigen.[15]

E. coli serotypes that cause disease or food poisoning are generally designated as interovirulent *E. coli* (EEC) and can be divided into six subgroups.[2, 13, 15] These include: Enteropathogenic *E. coli* (EPEC), Enterohemorrhagic *E. coil* (EHEC), Enteroaggregative *E. coli* (EAggEC) and diffusely adherent *E. coil* (DAEG). However, the last two groups are not well-studied.[16]

CHARACTERISTICS OF *E. COLI* O157:H7

Growth Requirements

Temperature: *E. coli* O157:H7 grows best within a temperature range of 30^0-42^0 C, the optimum temperature being 37^0 C.[2] The organism does not grow well at 44^0 C-45.5^0 C.[17] Most standard detection procedures for fecal coliforms are conducted in this higher

temperature range therefore fail to detect, the pathogen. It is capable of withstanding very low storage temperatures including frozen storage. Doyle and Schoeni[17] reported that *E. coli* O157:H7 in ground beef was held at-20^0 C for a month with very little reduction in number. Circumstantial evidence suggests that *E.coli* O157:H7 strains increase their thermal resistance at time of storage at -18^0 C increased. [15]

P^H Value: *E. coli* O157:H7 is capable of growing over the pH range of 4.4-9.0. However; there is a need of interaction with other factors including temperature, water activity and the nature of the acidulant, for this strain to be grow. [2]

Water Activity (a_w): The minimum water activity level for the growth of *E. coli* O157:H7 is 0.95. [1, 2]

Type of Toxins Produced and Their Actions

A distinctive feature of *E. coli* O157:H7 is the production of large quantities of Shiga-like toxins (SLT) or Vero toxins (VT).[5] SLTs are a family of cytotoxic proteins that consists of an approximately 32 KDa A subunit, with N-glycosidase activity, noncovallently associated with a pentamer of B subunits, which mediate binding to specific receptor molecules. The major *E. coli* O157:H7 SLTs consists of LT 1, which is identical to *Shiga toxin* of *Shigella dysenteriae*, and SLT-2, which is about 60% homologous to SLT-1. [7] In addition, there are variants of SLT-2 identified as SLT-2c, SLT-2d, SLT-2e, and SLT-2f. SLT-2e is typically found as the only SLT in porcine edema disease strains of SLT-producing *E. coli*, where as SLT-2f is found in *E. coli* isolated from healthy pigeons. Combination of SLT-1, SLT-2, SLT-2c, and SLT-2d are found in *E. coli* that are carried by healthy ruminants and implicated in diseases in humans.[18] Both toxins are encoded by lysogenic bacteriophages. [7, 18, 19]

Typically binds with, SLT high affinity to its glycolipid receptor, globotriaosylceramide (Gb_3), at the surface a host epithelial or endothelial cell, permitting the toxin molecule to be internalized by receptor-mediated endocytosis. [20] Following retrograde transfer through the Golgi -apparatus, the toxin becomes associated with the rough endoplasmic reticulum, from which it is released into the sites. During transport of SLT, cleavage of the A sub-unit by the enzyme furin and reduction of a di-sulfide bond result in separation of a small A_2 fragment from the 27 KDa A_1 fragment. The A_1 fragment interacts with 28s r-RNA of 60s ribosome and catalyzes the removal of a specific adenine residue, thereby inhibiting protein synthesis.

Inhibition involves peptide chain termination at the stage of aminoacyl-t-RNA binding to the acceptor site on the ribosome. This activity can be lethal for the host cell. [7, 18, 19]

Salt Tolerance

E. coli O157:H7 at 4.5%Nacl in broth shows a threefold increase in generation time, whereas at 6.5%, a 36- hour lag was noted with a generation time of 31.7 hours. No growth occurred at ≥8.5%NaCl. Similarly *E. coli* O157:H7 survived sausage fermentation but did not grow when stored at 4˚c for two month following incubation at a level of 4.8×10^4 cfu/ml. [21]

Thermal Resistance

The thermal resistance of *E. coli* O157:H7 is like that of most gram-negative bacteria, and in fact, these stains appear to be more heat sensitive than most Salmonella.[21] Although *E. coli* O157:H7 cells become more heat sensitive in apple juice when L-malic acid was increased from 0.2-0.8%, or when pH was reduced from 4.4-3.6, benzoic acid at 1000ppm was the most effective additive in increasing heat sensitivity.

Acid Tolerance

E. coli O157:H7 is relatively acid-tolerant and may survive for weeks at pH 4.2, there by enhancing its ability to survive in food and in the environment. It can survives a wide pH range of 4.0-9.0 and even survives at a level as low as 2.0 and as high as 11.0 [2]. The O157 (and certain other serotypes of STEC) are exceptionally acid resistance in vitro being able to survive exposure to pH 2.5 for over 3 hrs, but there is considerable variation in acid resistance among strains of EHEC.

At least three acid resistance mechanisms have been identified in E coli O157:H7: a glutamate dependent system, acid inducible arginine-dependent and oxidative system.[1] In addition, the O polysaccharide has been reported to contribute to acid resistance. The acid resistance of *E coli* O157:H7 is responsible for enhanced survival in acidic foods and resistance to killing by HCl in gastric juices, and may be related to a low infection does for humans. The O157:H7 strain also carries one or two copies of genes for urease production. These genes are not expressed in vitro, but they are functional and may contribute to acid resistance in vivo.[1]

Response to Antibiotics

E. coli O157:H7 is resistant to many of the antimicrobials. The prevalence of antimicrobial resistance among isolates of *E. coli* O157:H7 recovered from clinical cases in humans, pigs, cattle and food over a 15-years period (1985-2000) in the USA has been described. There was a high prevalence of resistance to tetracycline, sulfamethoxazole, cephalothin, and ampicillin. The highest prevalence occurred among isolates from pig more than 50% of isolates were resistant to sulfamethoxazole, cephalotin, or tetracycline and more than 20% were resistant to ampicillin or gentamicin.[13]

EPIDEMIOLOGY OF *E. COLI* O157:H7

Occurrence in Animals

Based on outbreaks in the USA, studies were conducted to evaluate the infection rate in cattle. The agent, were isolated from only 25 suckling calves of the approximately 7,000 examined in 28 states.[16] This study indicated that *E. coli* O157:H7 is widely distributed in USA, but that the proportion of animals harbouring this serotype is low. The prevalence of infected herds is estimated at approximately 5%. In Washington State, 5% to 10% of herds harbour *E. coli* O157:H7. This serotype was also isolated from cattle in Argentina, Canada, Egypt, Germany, Great Britain, and Spain. In Argentina and Spain, there was an association between serotype O157:H7 and a diarrheal disease in cattle, whereas in other countries the isolates were produced from apparently normal cattle.[16]

E. coli O157:H7 strain isolations were made mainly from calves and heifers, but also from dairy cows' especially young animals, and beef cattle.[2, 4] Calves infected with O157:H7 may become clinically ill, but mostly the animals harbour the organism in their intestines as unapparent carriers. The fact that *E. coli* O157:H7 is uncommon in adult cattle leads to the suggestion that the finding in meat would be caused mainly by fecal contamination from different sources.[4] However, contamination of meat from animals other than cattle is low.[16]

E. coli O157:H7 may colonize poultry. Isolations are known from chicken caeca and turkey meat.[4]

Occurrence in Humans

Human infections with *E. coli* O157:H7 has a world wide distribution. Serotype O157:H7 has been isolated in outbreaks in Canada, Great Britain, and the United States. It has also been isolated in Argentina, Belgium, the former Czechoslovakia, and China.[8] These isolates were

obtained from fecal samples taken from sporadic cases of hemorrhagic diarrhea submitted to public health or hospital laboratories for examination.

In the United States, where the first case confirmed in 1982, population surveys made in 1985-1986 led to an infection rate of 2.1 per 100,000 inhabitants annually. There the infections have been concentrated in the northern states.[4]

From 1982 to 1992, 17 outbreaks occurred in the USA; the smallest affected 10 people and the largest, 243. In November 1992, an outbreak occurred among people who had eaten undercooked hamburgers at a fast food restaurant chain. The same E. coli serotype was isolated from the ground beef found in these restaurants. Seventeen more outbreaks occurred in 199.[16] Case reporting is now compulsory in 18 US states. It is estimated that there are 8 cases each year per 100,000 inhabitants in Washington D.C. Approximately the same incidence as for Salmonellosis.[16] During the same period (1982-1992) there were 3 outbreaks in Canada and two in Great Britain.[8]

E. coli O157:H7 has been the cause of hemorrhagic diarrhea in 15% to 36% of the cases in the US and in 39% of cases in the United Kingdom. Sporadic cases of hemorrhagic colitis and of HUS were reported in Germany and in other, mostly northern countries.[4]

In a cross-sectional prevalence study of *E. coli* O157:H7 in 384 diarrheic patients in teaching hospital of university of Gondar, Ethiopia by using latex agglutination test from stool sample found no positive sample for the bacteria.[9]

Sources of Infection

Cattle appear to be the main reservoir of *E. coli* O157:H7. Infected persons, animals including sheep, goats, pigs and poultry are also reservoirs. Transmission to human is principally through the consumption of contaminated foods, such as raw or undercooked meat products and raw milk. A retail meet study conducted by Doyle and Schoeni[17] indicated that *E. coli* O157:H7 is isolated from 1.5% pork, 1.5% poultry, and 2.0% of lamb samples tested indicates that the organism may be associated with foods of animal origin in addition to beef. Fresh pressed apple juices or cider, yoghurt, cheese salade vegetables and uncooked corn have also been implicated. Fecal contamination of water and other foods, as well as cross-contamination during food preparation may also be responsible.[2]

In one outbreak, this occurred in Cabool, Missouri (USA) in 1989 that affected 243 people (one of every 12 people in the town), was caused by city-supplied water. The water has been believed that contaminated by deer feces.[16]

In a few cases, *E. coli* O157:H7 was transmitted by direct contact, with infected cattle or horse to humans.[7] Human-to-human transmission, mostly by direct contact is not rare in infant wards, kindergartens, nursing homes, and families. Accordingly, a baby-sitter contracted the infection while carrying for a sick child. Furthermore, secondary cases also occurred in day-care centers.[7]

Mode of Transmission

Pathogenic *E. coli* O157:H7 strain largely transmits through ingestion of contaminated food, mainly unpasteurized milk, hamburger, apple juice and water; fecal-oral-route; and person-to-person transmission or contact.[2, 16]

THE DISEASE PATHOGENESIS

E. coli O157:H7 cause HC, HUS and TTP, but the mechanism of pathogenesis is not yet fully understood.[2]

E. coli O157:H7 strains are characterized by the presence of SLT genes, locus for enterocyte effacement (LEE) and a high-molecular-weight plasmid that encodes for a hemolysin. These three virulent factors are responsible for the pathogenesis of the disease associated with bloody diarrhea and hemolytic-uremic crisis in humans.[3]

The LEE is a large cluster of genes that are collectively responsible for the intimate attachment of the bacterium to the apical membrane of enterocyte and subsequent destruction or effacement of the microvilli.[2]

E. coli O157:H7 also possesses a high-molecular-weight plasmid that contains several putative virulence genes, including a pore-forming homolysin.[2]

SLTs, which are secreted by the bacteria colonizing the colon, pass through the intestinal wall via transcytosis and subsequently spread hematogenously to target tissues by causing damage and swelling to the endothelial cells lining the blood vessels. Traveling through the damaged, swollen glomerular capillaries, red blood cells can be destroyed (hemolysis) or fragmented. Platelets also clog these capillaries.[2] Ultimately, the toxins preferentially attach the renal

tissue because of their affinity for receptors that are found predominantly in this tissue. After internalization by receptor-mediated endocytosis, the active subunit of the toxin catalytically inactivates ribosomes by cleaving a residue on the 28s r-RNA [4, 18]. By impairing filtration, this phenomenon can lead to kidney failure.[2] Cell death within the glomeruli consequently results in tissue damage that can be further complicated by the ensuring inflammatory responses.[4]

THE DISEASE CHARACTERISTICS

Infectious Dose

The infective dose for *E. coli* O157:H7 is not well known. However, a complication of outbreak data indicates that it may be as low as 10 organisms which is similar to that of *Shigella spp.* Estimates derived from an outbreak due to dry-cured salami suggested that does between 5 and 50 organisms were effective in causing bloody diarrhea.[5] Such a low infective dose has given rise to the view that this organism is usually tolerant of the stresses that are used by the body as defense mechanisms. Therefore, low number, up to 10,[5] of the organism is required to cause illness especially in young children, the elderly and immune-compromised persons. The lower infectious dose of *E. coli* O157:H7; therefore, underscores the importance of proper decontamination procedures since very low levels of injured cells could possibly recuperate and cause illness.[2]

Susceptible Population

Susceptibility to *E. coli* O157:H7 infections follows the usual pattern of the old, the young and those with underlying infections (immune compromised) being at greater risk.[15]

Although the epidemiology of EHEC strain is not fully established, it would appear that those at extremes of age, children aged under 15 years and geriatrics, are more susceptible. HUS is most common in children under 16 years of age and in the elderly. This may reflect host adaptation by SLT-2 producing strains, which are rare in patients aged 17 to 61 years.[15]

Risk of EHEC infection is also increased by gasterectomy, prior antibiotic therapy, and H-blocking agents. Antibiotic therapy in the course of the disease can lead to HUS.[15]

The Disease in Animals

Animals are known to carry *E. coli* O157:H7, but significant clinical diseases are seldom reported, in contrast to those due to other *E. coli* serotypes. This *E. coli* form may be a source for diarrhea in cattle.[4]

Animal models have been used to study the pathogenesis of *E. coli* O157:H7. After inoculation, this organism induce non-bloody diarrhea in infant rabbits, 2-week-old guinea pigs, 3-week-old mice, and young rhesus monkeys. Infant rabbits inoculated with 10^8cfu developed watery diarrhea 3-7days after inoculation. With increasing age the susceptibility of rabbits decreased; older rabbits failed to develop diarrhea. Adult mice are sensitive to cytotoxins of *E. coli* O157:H7. SLT-1 infected intraperitoneally led to leg paralysis and death. Neonatal pigs inoculated perorally with 10cfc E. coli O157:H7 developed anorexia, lethargy, and watery diarrhea.[4]

In rabbits, piglets, and other laboratory animals, lethal effects of Shiga-like toxins are known, but these animals do not develop typical symptoms of HUS and HC.[4]

The Disease in Humans

The incubation period of *E. coil* O157:H7 infection lasts form 2-9days (average 3-4 days). The disease is associated with sever complications, such as HUS, HC and TTP in humans. The appearance of the disease ranges from a slight case of diarrhea to severe HC, with strong abdominal pain and little or no fever. At the out set, diarrhea is watery but later becomes hemorrhagic, either with traces of blood or highly hemorrhagic stool. Diarrhea lasts an average of 4 days and about 50% of patients experience vomiting. Hemorrhagic diarrhea was present in more than 95% of a large number of sporadic cases recorded.[16]

HUS is leading cause of kidney failure in children, which often requires dialysis and may ultimately be fatal. In HUS the patient suffers from bloody diarrhea, hemolytic anemia, kidney disorders and renal failure. 2-7% of patients (up to 30% in certain outbreaks) will progress to HUS and subsequent, complications. The mortality rate is 3-17%. Insufficient red bleed cells (hemolytic anemia, low blood platelet count (thrombocytopenia) and sudden, marked decrease in kidney functions (acute renal failure) remark HUS and nearly always occur after several days of diarrhea that's blood.[2]

Another complication is TTP, which is characterized by thrombocytopenia, hemolytic anemia, and azotemia, fever thrombosis in the terminal arterioles and capillaries, and neurological symptoms that dominate clinical picture. Blood clot in the brain may occur, frequently resulting in death. An additional risk factor seems to be treatment with thrimethoprim-sulfamethoxazole or gentamycin during the syndrome. These antibiotics kill verotoxigenic E coli but liberate the toxin without deactivation.[16]

DIAGNOSIS OF *E. COLI* O157:H7

Culture and Isolation of *E coli* O157:H7

Fecal shadings of *E. coli* O157:H7 in cattle often occurs at levels lower enough that selective enrichment and Immunomagnetic Separation (IMS) are required for detection. Sorbitol MacConkey agar(S-MAC) agar containing cefixime and tellurite is the standard planting method for the isolation of the organism.[1]

Selective Enrichment

Many laboratories use a special type of MacConkey agar to screen for *E. coli* O157:H7. S-MAC containing sorbitol instead of the lactose present in routine MAC agar. Because *E. coli* O157:H7 does not metabolize sorbitol, it produces colorless colonies on S-MAC. Most other *E. coli* strains ferment, sorbitol and therefore, produce red colonies on S-MAC.[22]

Different researchers; however, found non-sorbitol-fermenting organisms, which primarily include non-SLT-producing *E. coli* strains, *proteus spps.*, and *Aeromonas spps*., in human fecal specimens. To distinguish *E coli* O157:H7 from these other non-sorbitol fermenters on S-MAC plates, rhamnose is added at a concentration of 0.5 ppm. In contrast to 60% of other non-sorbital fermenting *E. coli* strains, *E. coli* O157:H7 isolates do not ferment rhamnose rapidly. To inhibit *proteus spps.*, cefixim was added at 0.05 µg/ml concentration. On the other hand, to inhibit providencia *spps.* and *Aeromonas spps*. which are prevalent, in the feces of both humans and cattle, potassium tellurite was added at 2.5µg/ml, to create CT-SMAC. Furthermore, absence of ß-Gulucoronidase activity is a consistent trait of *E. coli* O157:H7 strain.[22]

Immunomangnetic Separation (IMS)

This method employs magnetizable beads (about 2-3 µm in size), about 10^6-10^8/ml that are coated with antibody by incubating in the refrigerator for varying periods of time up to 24 hrs. The unabsorbed antibody is removed by washing. When properly treated, the coated beads are added to food slurry that contains the homologous antigen (toxin), thoroughly mixed, and allowed to incubate from a few minutes to several hours to allow for reaction of antigen with antibody coated beads. The antigen-antibody complex is collected by a magnet followed by elution of antigen or measurement on beads. The concentrated antigen is assayed by other methods. [21]

In a recent study, IMS was combined with flow cytometry for the detection of *E coli* O157:H7. The antigens were labeled with fluorescent antibody, which was measured by flow

cytometry, and the combined method could detect <10^3 cfu/g of pure culture or 10^3-10^4 cfu/g in ground beef. This method may be also used for a number of other organisms including viruses and protozoa. [21]

Latex Agglutination Test

In the late 1980s, latex agglutination reagents for the detection of *E. coli* O157:H7 antigens became available commercially and were shown to be a rapid and economical alternative to tube agglutination [3, 21]. However, this method requires precaution to control false-positive identifications of *E. coli* O157:H7. [21]

Sorbitol negative colonies selected from S-MAC will be tested with *E. coli* 0157 antiserum or latex reagents (O157 antibody-coated latex and control latex) for the isolation and identification of *E. coil* O157:H7. If using O157 latex reagents, it is important to test, isolates in the control latex to detect nonspecific agglutination of organisms with latex. It is recommended to heat strains that agglutinate in the latex control reagent and then retesting them in both the O157 antibody-coated and control latex reagents. However, *E. coli* O157:H7 strains have not been shown to agglutinate in both the antibody-coated and control latex reagents.[2]

Biochemical Test

The O antigens of several bacterial *spps.*, mainly *Salmonella, Yersina enerocolitica, citrobacter freundii and E. hermanii* are known to cross- react with *E coli* O157:H7. Hence presumptive O157:H7 isolates should be identified as *E coli*. Because *E. hermanii* cross-reacts serologically with O157:H7, is biologically very similar to *E. coli*, and is sorbitol negative, special precaution should be taken to differentiate the two organisms. *E. hermanii* can presumptively be differentiated on the basis of the appearance of its colonies, which are gold colored. Further definitive differentiation of *E. coli* and *E. hermanii* can be made by using one or both of the two tests: growth in the presence of potassium cyanide /KCN/ and fermentation of cellobiose. *E. coli* O157:H7 (and other *E. coli*) cannot grow in the presence of KCN and does not ferment cellobiose, where as *E. hermanii* is found positive in both these tests.[1]

Direct Immunofluorescence (DIF) and Enzyme Linked Immunosorbent Assay (ELISA) Methods for the immunological detection of *E. coli* O157:H7 antigens directly in the feces

have been developed. The main incentive for development of these tests was to provide a more rapid diagnosis than can be made by culture. [1]

A DIF assay was developed and evaluated for the detection of *E. coli* O157:H7 in human feces.[1] The DIF was tested on fecal smears that were either treated or not with bleach. The fecal smears were then stained with commercial fluorescein-conjugated anti-*E. coli* O157 polyclonal antibodies and examined microscopically. The DIF assay detected all (336) isolates of *E. coli* O157:H7 that were recovered by culture. No false- negative results were obtained with bleach pre-treated specimens. The turnover time for DIF assay was less than 2 hrs.[1]

ELISA is an immunological method that employing an enzyme coupled to either a solid-phase (polystyrene) coated with antigen or antibody. The ELISA technique is used in the detection of *E. coli* O157:H7 enterotoxins. A monoclonal antibody specific for EHEC stains was shown to be highly specific when used in an ELISA to detect EHEC stains. Two "Sandwich" ELISA were developed based on toxin-specific murine monoclonal cupture antibodies and rabbit polyclonal second antibodies specific for the SLT-1 and SLT-2 genes of *E. coli* O157:H7. The SLT-1 ELISA could detect 200g of purified SLT-1 toxin, whereas the SLTS-2 could detect 75pg of SLT-2 toxin.[21]

In another study, a competitive ELISA was compared to a DNA probe and the suckling mouse assay for E. coil SLT-1 enterotoxins. The probe was more specific, but ELISA was more sensitive and the most rapid of the three methods.[1, 21]

According to Jay [21] an ELISA was compared with culture for the detection of *E. coli* O157:H7 in human fecal specimens. This assay utilized plastic micro-well test strips coated with anti- *E. coli* O157:H7 polyclonal antibodies and detect *E. coli* O157:H7 antigen. The ELISA was found to be an acceptably sensitive, specific and rapid method for directly screening stool samples for *E. coli* 0157:H7 and required only about 1 hr to complete. However, ELISA was unable to distinguish toxigenic from non-toxigenic strains of *E. coli*.

Polymerase Chain Reaction (PCR)

By employing thermostable DNA polymerases about $5^{'}$ and $3^{'}$ specific oligonucleotide primers, a single molecule of DNA can be amplified to 10^{7} molecules after a serious of amplification cycles typically from 20 to 50. The amplified DNA is then detected by use of

either agarose gels or southern hybridization employing either radio labeled or non-radiolabeled probes. [21]

E. coli O157:H7 assay is a PCR-based method that uses tableted reagents, and when compared with other methods for detecting low levels of the organism (<3/g) in ground beef, it detected 96.5% of positives compared to 71.5% for immunoassay methods and only 39% for the best culture method.[21]

PCR has been used both to confirm the identity of *E. coli* O157:H7 isolates and to directly detect the organism in feces or enrichment broth cultures. Because of problems caused by PCR inhibitors in feces, most PCR assays have been used to confirm isolates as *E. coli* O157:H7; however, even for this purpose, most methods have had pitfalls. Collectively different studies suggest that PCR or multiplex PCR can potentially be applied to enrichment cultures for rapid, sensitive, and specific detection of *E. coli* O157:H7 in bovine feces. Multiplex PCR has shown to be very useful for confirmation isolates as *E. coli* O157:H7.[21]

Multiplex or collective individual PCR reactions targeting the *E. coli* O157:H7 antigen gene cluster (such as rfb E), H_7 antigen (fli C), SLT-1 and SLT-2, and intimin (eaeA) on suspect isolates are recommended for confirmation as *E. coli* O157:H7.[1]

TREATMENT

E. coli O157:H7 infection has no specific immunizations and prophylaxis so far in use. As a result, therapy is primarily based on fluid and electrolyte replacement. Antibiotic treatment is contraindicated since it does not alleviate any symptoms, contributes to verotoxin release, and increases the risk of HUS.[2] However, in severe cases antibiotics may be administered.[2, 7] Motility inhibiting drugs are also contraindicated since they lead to retention of EHEC and increase the possibility of Vero-toxin absorption.[7]

Patients with HUS and TTP must be treated in intensive care units since early dialysis, control of electrolytes, treatment of hypertension, and blood exchange transfusion may be necessary. Improved clinical management and renal dialysis have reduced the mortality rate to <10%. However, approximately 30% of the survivors suffer from permanent, disabilities e.g., chronic renal insufficiency, hypertension, and neurological deficits.[7]

PREVENTION AND CONTROL

The prevention and control of *E. coli* O157:H7 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 establishment and domestic environment including general hygienic measures.[3]

Infection can be prevented through avoiding eating of raw or undercooked beef, avoiding drinking unpasteurized milk or milk products, or fruit juices, thorough washing of hands regularly after bowel movements, before and after food preparation and following contact with cattle and their excreta.[2] Washing of all vegetables and fruits thoroughly and drinking of municipal water that has been treated with adequate levels of chlorine or other effective disinfectants is important. In addition, pasteurization of fruit juices and milk, and cooking of meat, with moist heat (121^0c for at least 15min) and dry heat ($160\text{-}170^0$c for at least 1 hr) as well as irradiation are effective methods for the control of the infection.[3] Other preventive measures include good hygienic practices when handling diapers at day care centers to decrease secondary person-to-person transmission.[1]

Vaccination of cattle and administration of 1% sodium chlorate in feeds of cattle has been investigated to have a significant effect in reducing *E. coli* O157:H7 shedding. There is evidence that virulent factors secreted by the type II system can be used as effective vaccine component for the reduction of colonization of cattle by *E. coli* O157:H7.[3] However, now a day, it is impractical.[7]

CONCLUSIONS

The emergence of *E. coli* O157:H7, with its very low infectious dose and associated risk of serious human illness, has greatly increased the potential for zoonotic disease acquired from livestock. *E. coli* O157:H7 causes a wide spectrum of illnesses in humans. These range from asymptomatic carriage to HC. Complications such as the HUS and TTP can cause substantial morbidity and mortality in humans. HUS and TTP are serious illnesses characterized by kidney failure, and destruction of the red blood cells leading to anaemia, respectively. Young children and the elderly are at greatest risk of developing HUS or TTP following *E. coli* O157:H7 infection. Cattle being the main reservoir of *E. coli* O157:H7, beef and milk are important source of infection. Transmission to humans is principally through the consumption of contaminated foods, such as raw or undercooked meat products and raw milk. Therefore, having the above conclusions in mind, the following points forwarded as

recommendations: Awareness should be created among consumers as to the importance of hygienic practices in the preparation, handling and storage of food items in the control and prevention of this newly emerging pathogen. There should be an implementation of effective management procedures extending from the farm environment, slaughtering process, retail handling and processing to ultimately the consumer for the control and prevention of the disease. The public should especially be aware about the importance of cooking all ground beef and make sure that any juice is clean and the inside is hot to prevent the transmission of infection. Studies must be done about the epidemiology of the bacteria in animals in Ethiopia, where eating raw meat is a culture, to estimate the public health risk associated with this disease.

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