DIVERSITY IN *ESCHERICHIA COLI* O157:H7 BETWEEN HUMAN AND BOVINE STRAINS

by

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Abstract

Within the United States, it has been estimated that 60 deaths and 73,000 illnesses are caused by *Escherichia coli* O157:H7 infection annually (Gavin et al., 2004). Multiple effects have been known to occur with the onset of infection from *E. coli* O157:H7 in which some of these can become life-threatening. *Escherichia coli* O157:H7 is defined as a Shiga-toxin-producing *E. coli* strain (STEC). This microbial pathogen is a gram-negative bacillus organism that is motile, non-sorbitol fermenting, and β-glucuronidase negative. The infectious dose of *E. coli* O157:H7 can be as low as ten cells (Food and Drug Administration, 2009).

Consumption of contaminated food, mainly undercooked ground beef and non or incorrectly pasteurized milk, are the primary sources of *E. coli* O157:H7 infection in human. Cattle, in particular, are considered chief asymptomatic reservoirs for this pathogen. Carried in their gut, feces, and milk, cattle carry this Shiga toxin-producing *E. coli* in ranges from 10^2 to 10^5 CFU/g. Although colonized with *E. coli* O157:H7, cattle and other ruminants show no adverse side effects from the pathogenic bacteria. There is also a difference in the prevalence of this pathogen between human and cattle. There has been a low incidence of illness caused by *E. coli* O157:H7 in humans when compared to the high prevalence of *E. coli* 057:H7 found in cattle and their environment.

It has been discovered, through population genetic analysis, that *E. coli* O157:H7 and other O157:H^- isolates make up a clone complex. In spite of the clonal nature of *E. coli* O157:H7 and other O157:H^- isolates, there are significant characteristics showing variability between the clone complex. These variability aspects can possibly account for the rapid divergence of *E. coli* strains including the recently discovered divergence of *E. coli* O157:H7 in to two separate lineages. Other possible reasons for a non-linear relationship between cattle prevalence and human infection include diversity of the Shiga Toxin-Encoding bacteriophage and receptors in cattle verses human, and finally the difference between the production of Locus of Enterocyte Effacement (LEE) in both human and cattle lineages.

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Acknowledgments

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Dedication

I would like to dedicate this report to Sean and I's future daughter. This report topic became an option only after being informed of my pregnancy. Since then, I have gained more interest in this field of microbiology and wish to participate in laboratory research within this area in the future.

CHAPTER 1 - Introduction of *Escherichia coli* O157:H7

History and General Knowledge of *Escherichia coli* O157:H7

In 1983, a microbial investigation was carried out by state health departments and the Centers for Disease Control and Prevention (CDC) to determine the cause of two hemorrhagic colitis outbreaks in 1982 in Michigan and Oregon. Stool specimens were compared to the only isolate of *Escherichia coli* O157:H7, at the time, a rare strain of *E. coli* stored at the CDC in Atlanta that was recovered in 1975 from a woman with bloody diarrhea. Matching the isolate from the CDC, it was confirmed that the stool samples contained *E. coli* O157:H7 (Tarr, 1995). In 1987, "Shiga-like toxin", similar to that of Shiga toxin produced by *Shigella*, was discovered in *E. coli* O157:H7 isolates, which helped define its pathogenic ability. These toxic effects became more publically known after they were involved with the largest US *E. coli* O157:H7 outbreak in 1993: Jack-in-the-Box outbreak. Increased screening for this pathogen in clinical laboratories has taken place and the US incidence of infection has risen remarkably since this outbreak. According to the CDC (CDC, 2009), this lethal pathogen was also responsible for the following outbreaks: infected ground beef sold at Kroger in 2008, a multi-state outbreak in ground beef patties in 2006, Taco Bell outbreak in 2006, and a recent outbreak in raw Nestle cookie dough in 2009. Throughout the decades, *E. coli* O157:H7 has caused numerous foodborne outbreaks and a rise in public concern due to its toxigenicity and repulsive effects in the human. Not only is *E. coli* O157:H7 now recognized as a common bacterial cause of bloody diarrhea, but also as the cause of Hemolytic Uremic Syndrome (HUS) in the United States, Europe and Canada (Besser et al., 1999).

Escherichia coli O157:H7 is defined as a Shiga-toxin-producing *E. coli* strain (STEC) and it has been suggested that this strain evolved from Enteropathogenic *E. coli* (EPEC) that acquired the Shiga toxin gene (Wachsmuth et al., 1997). This pathogen is a Gram-negative bacillus that is motile, and differs from other *E. coli* in being non-sorbitol fermenting, and β-glucuronidase negative. However, *E. coli* O157 isolates that are non-motile, sorbitol fermenting, and β-glucuronidase positive have been isolated in Germany and Australia (Kaper and O'Brien, 1998), but are rarely found in the United States. *Escherichia coli* O157:H7 is a specific isolate of the bacteria, *E. coli*. The O refers to the somatic antigen and the H refers to the flagellar antigen. Part of this strains pathogenic ability derives from the 157st O antigen in combination with the 7th H antigen and it is these two antigens that are targeted when *E. coli* O157:H7 is screened for. Possible signs of *E. coli* O157:H7 infection include, but are not limited to nausea, vomiting, slight fever, abdominal pain, HUS, thrombotic thrombocytopenic purpura (TTP), renal failure, lethargy, seizures, and coma (Su and Brandt, 1995).

Disease in Humans and Sources of Infection

In the United States, the most abundant amount of isolates have been recovered from Washington State, Oregon, Minnesota, and Massachusetts, suggesting that predominance of infections are in northern latitudes (Su and Brandt, 1995). According to the Center for Science in the Public Interest Database (2009), predominance of *E. coli* O157:H7 infections from 1990-2006 occurred in the contiguous northern states of North America (Fig. 1.1). Within the United States, it has been estimated that 60 deaths and 73,000 illnesses are caused by *E. coli* O157:H7 infection annually (Gavin et al., 2004). Multiple effects have been known to occur with the onset of infection from *E. coli* O157:H7 in which some of these can become life-threatening. Characterized by microangiopathic hemolytic anemia, thrombocytopenia, and renal failure, HUS

is a critical syndrome present in nearly 10 percent of children infected with *E. coli* O157:H7 (Tarr, 1995). HUS occurs predominately in young children and the elder and is the most common cause of acute renal failure in children. Required dialysis is needed for roughly half of patients with HUS, while three-quarters require transfusions of erythrocytes or dialysis and approximately 15 percent of HUS patients suffer from chronic renal failure or death (Tarr et al., 1989, Robson et al., 1991).

In addition to HUS, central nervous system complications, including blindness, seizures, and mental retardation, can occur in 30-50 percent of patients infected with *E. coli* O157:H7 (Cimolai et al., 1992) . The case - fatality rate among nursing home residents can be as high as 36 percent due to, in part, underlying diseases (Su and Brandt, 1995). In a large former nursing home outbreak with *E. coli* O157:H7, 17 to 19 residents died due to hemorrhagic colitis, pneumonia, and HUS (Carter et al., 1987). In non-life-threatening situations, individuals have been known to experience gastrointestinal complications, dehydration and severe abdominal pain due to bloody diarrhea. Within 2 to 3 days medical care is usually sought with a majority of patients recovering within 10 days of the symptom's onset (Riley et al., 1993).

The infectious dose of *E. coli* O157:H7 can be as low as ten cells. Consumption of contaminated food, mainly undercooked ground beef and non or incorrectly pasteurized milk, is the primary source of *E. coli* O157:H7 infection. Cattle, in particular, are considered chief asymptomatic reservoirs for this pathogen. Carried in their gut, feces, and milk, cattle can contain this Shiga toxin-producing *E. coli* in ranges from 10^2 to 10^5 CFU/g. Ground meat should be cooked to 165°F in order to ensure that *E. coli* O157:H7 has been killed (USDA FSIS, 2006). In 1993, the largest outbreak of *E. coli* O157:H7 in the United States took place when 732 cases of illness, including 4 deaths of children, resulted from adulterated hamburgers provided by the

Jack-in-the-Box restaurant chain (Clark, 2005). Although cattle are considered the main reservoir for this pathogen, a limited quantity of *E. coli* O157:H7 of bovine origin is associated with human infections. Other common sources of infection include sheep, horses, dogs, wild birds, and houseflies (Ahmad and Zurek, 2006). STEC-infected animals normally do not show signs of disease and, by law, can still be included in food production. Consumption of this STEC- containing food has been identified as a major route of human infection in the United States as well as several other countries. Ground beef is not the only path of transmission, but also juice, unpasteurized apple cider, ranch dressing, sandwiches, and many other meats including salami and roast beef (Fig. 1.2). Fruits and vegetables have recently been reported to have *E. coli* O157:H7 causing an increase in the number of outbreaks. In 2006, a multistate outbreak of *E. coli* O157:H7 swept through spinach fields in which the spinach was later bagged and transported to grocery stores for consumer consumption. There were 205 confirmed illnesses from this outbreak including 3 deaths and 105 hospitalizations in which 30 percent of these patients developed HUS (California Food Emergency Response Team, 2007).

Escherichia coli O157:H7 has been able to enter the human population in various ways as shown by Figure 1.3. Besides contaminated food, *E. coli* O157:H7 has been isolated from wild animal feces and the environment (Beutin et al., 2007). Untreated recreational waters, such as lakes, are often populated with this pathogen due to contaminated animals feces entering the body of water. Individuals may swim in contaminated lakes and unknowingly become infected and possibly become a reservoir by person to person contact.

Highly resistant to physiochemical stresses, STEC strains are capable of living in acidic conditions as well as dryness. Surviving long periods of time in soil, manure, and water are also characteristics of this hardy pathogen. Not only is *E. coli* O157:H7 resistant to stress, but also to

antibiotics. Recently, it has been demonstrated that *E. coli* O157:H7 has become increasingly more resistant to streptomycin, sulfisoxazole, and tetracycline, possibly as a result of the prevalence of this organism in food animals that receive these antibiotics (Besser et al., 1999). Even though antibiotics relieve mild symptoms, there has not yet been an effective treatment in reducing HUS or the Shiga toxin-producing virulence factor emitted by *E. coli* O157:H7.

Virulence Factors of *Escherichia coli* O157:H7

The Shiga like toxin-producing element is the defining characteristic of STEC. Shiga toxins were first classified in *Shigella dysenteriae* and has became a part of an *E. coli* isolate after contacts between the two organisms. The life-threatening complications that result from *E. coli* O157:H7 infections are based on the production of virulence factors, Shiga toxin I (*stx*1) and Shiga toxin II (*stx*2). Shiga toxin is comprised of two subunits, AB_5 (Fig. 1.4). The A subunit is responsible for causing the toxic effect of the protein, while 5 similar B subunits all work together to bind to receptors on target cells (Cherla et al., 2003). Based on epidemiological data, isolates that produce *stx*2 alone are more likely to cause severe disease compared to isolates that produce only *stx*1 or the combination of *stx*1 and *stx*2, although, the latter two are capable of producing disease. Evidence that *stx*1 causes severe STEC infection comes from multiple animal studies. For example, after administered s*tx*1, intravenously, baboons produced clinical symptoms of HUS (Ritchie et al., 2003).

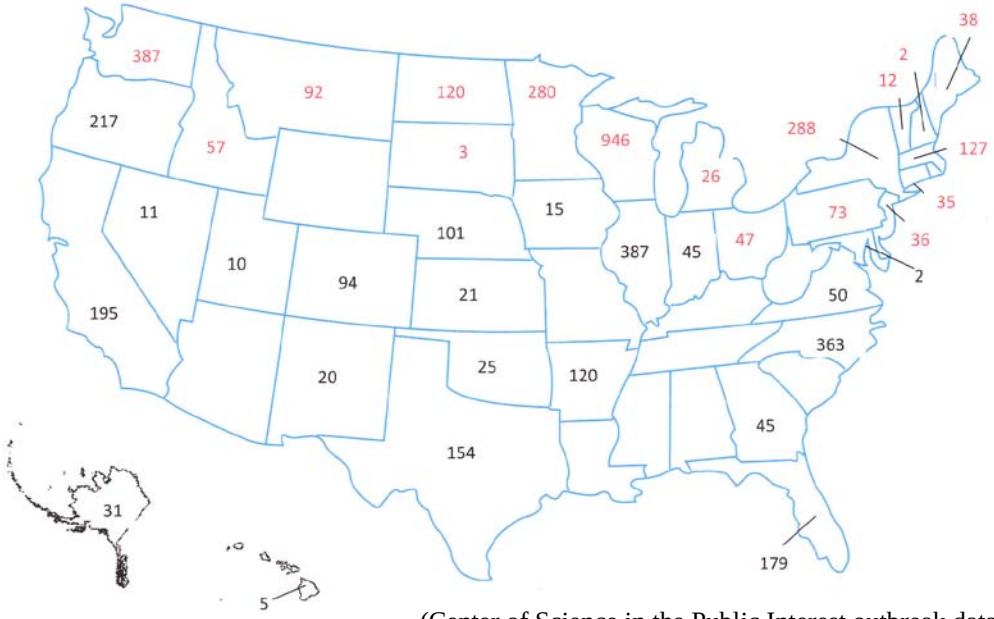
The virulence of *E. coli* O157:H7 is partly based on the prophage encoded Shiga toxin within the bacterium. Bloody diarrhea and abdominal cramps result from *E. coli* O157:H7 infections and are often initiated when endothelial cells in the digestive tract or kidneys are destroyed by the toxin. The severity of STEC infections correlate to the amount of bacterium

ingested and the amount of Shiga toxin produced. Unfortunately, low quantities of each can initiate an *E. coli* O157:H7 infection in humans.

Shiga toxin is not the only virulent factor in STEC. Intimin is a major concern as this is the adhesion molecule responsible for attachment of STEC organisms to the epithelial cells of the intestine, which can cause structural modifications and lesions. Finally, a third virulent factor in STEC organisms are called Locus for Enterocyte Effacement (LEE), which is a cluster of genes that encode various virulent factors making the organism pathogenic. One of these genes is the *eae* gene which is responsible for encoding the adhesion molecule, intimin. It is purposed that these three specific virulence factors work together to produce the pathogenic effects *E. coli* O157:H7 (Boerlin et al., 1999).

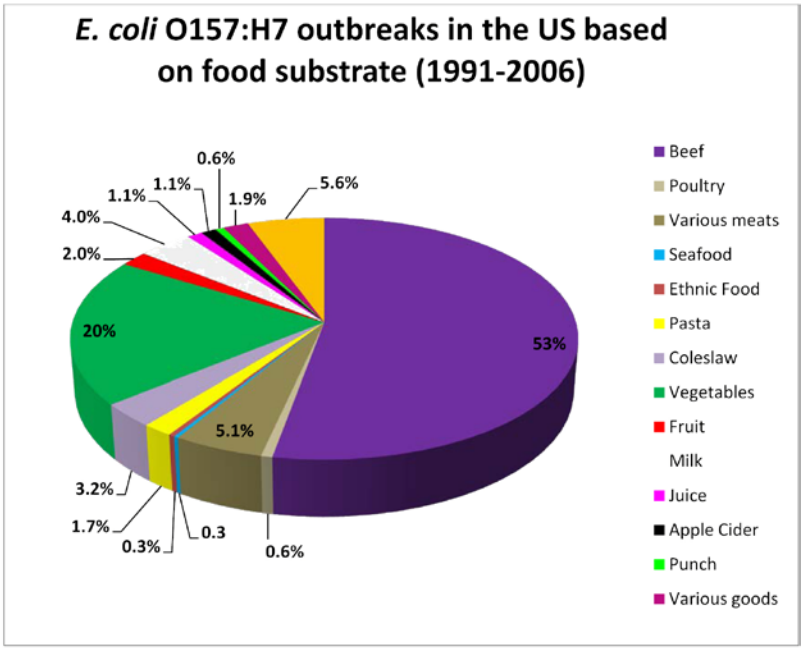
Figures and Tables

Figure1.1 Map representing number of *Escherichia coli* O157:H7 cases during 1990-2006. (Multistate outbreaks are not included in the numbers shown).



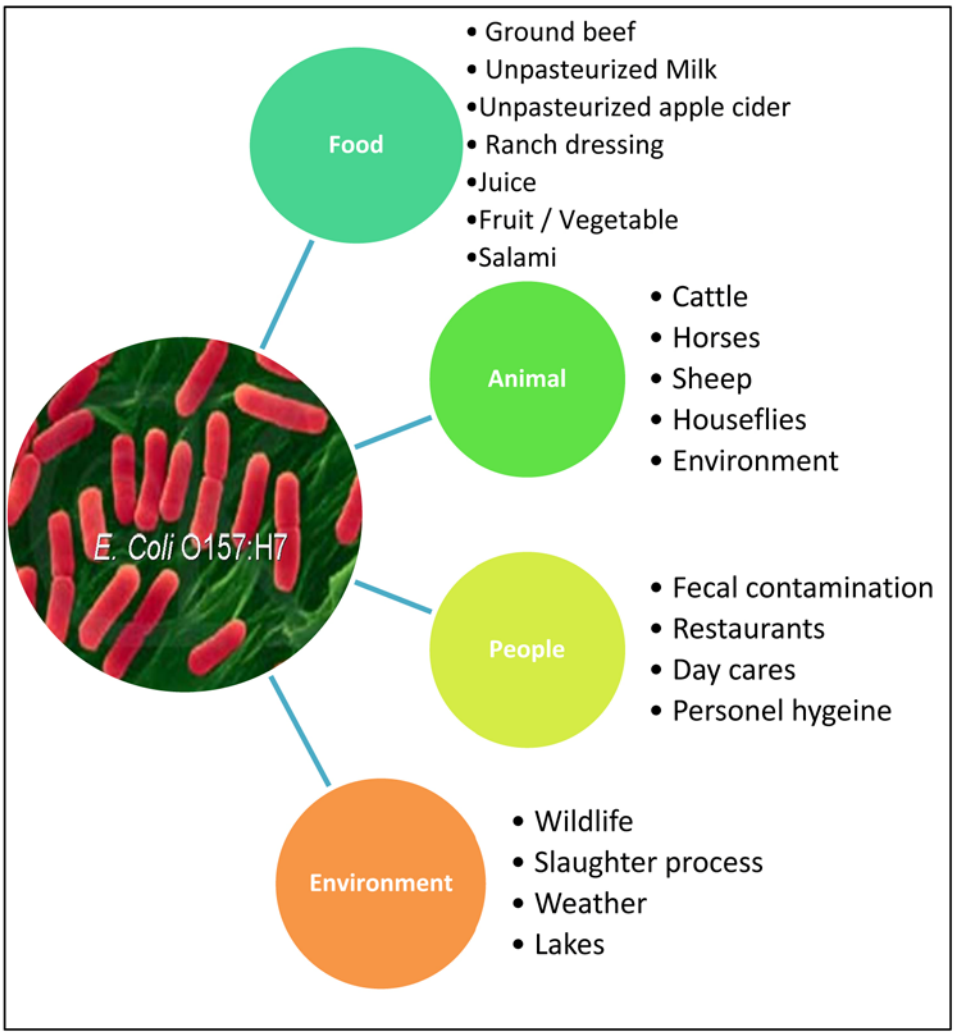
(Center of Science in the Public Interest outbreak database, 2009)

Figure 1.2 Percentages of *Escherichia coli* O157:H7 outbreaks based on contaminated food sources.



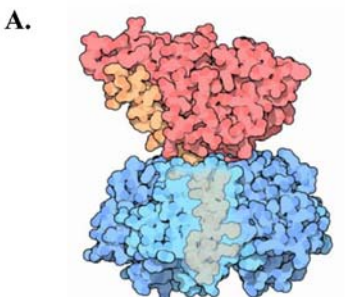
(Center of Science in the Public Interest Outbreak Database, 2009).

Figure 1.3 Possible routes of how *Escherichia coli* O157:H7 can enter the human population.

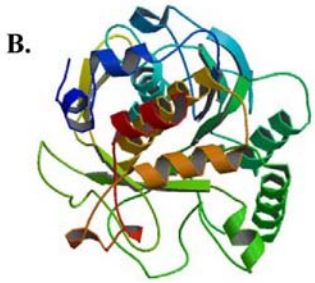


(Page, 2009)

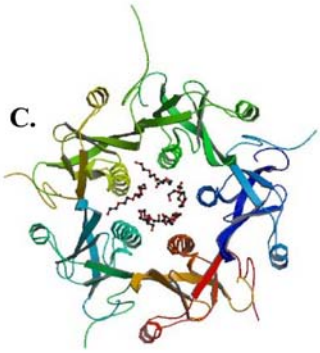
Figure 1.4 (A) General structure of an AB_5 toxin molecule. (B) The A subunit of the Shiga-toxin. (C) The B subunit from Shiga-toxin.



(Merrit and Hol, 1995)



(Paton et al., 2006)



(Byres et al., 2008)

CHAPTER 2 - The Presence and Prevalence of *Escherichia coli* O157:H7 in Cattle

Escherichia coli O157:H7 was introduced into the cattle population by the means of feed. Grain- fed cattle seem to have a significantly higher level of *E. coli* O157:H7 compared to grass-fed cattle. A grain diet turns a cattle's rumen into an ideal habitat for *E. coli* O157:H7 while the lethal strain of *E. coli* cannot survive for long periods of time on a grass fed rumen. Not only is there an increased number of *E. coli* O157:H7 in rumens of grain-fed cattle, but also in the tons of manure they produce each year. Approximately a billion tons of contaminated manure is produced, by agricultural animals, each year and often ends up in locations other than pastures, causing bacteria to travel to other habitats, animals, and environmental resources. Ultimately, this brings the pathogen closer to humans and finished food products with a chance of increasing infection rates in the human population (Pollan, 2006).

Several foodborne illnesses in humans result from the consumption of food products that are contaminated with pathogenic bacteria. Products can be contaminated via poor water sources, environmental agents, or other suitable hosts such as animals and insects. Even though safety policies such as HACCP (Hazard Analysis and Critical Control Points) and GMP's (Good Manufacturing Practices) have been put into effect, cattle have been identified as the primary source of *E. coli* O157:H7 in the United States. *Escherichia coli* O157:H7 is transmitted to human by the cattle in various ways including: contaminated cattle hide, the cattle's natural environment, drinking water, shedding of feces, agricultural fairs, and through cross contamination in the slaughter process. Seasons can have an influential effect on the prevalence of *E. coli* O157:H7 isolated from cattle.

It has been suggested by Omisakin et al. (2003) that the concentration of *E. coli* O157:H7 in ruminant feces increases during warmer months of the year, and this fluctuation of bacteria, during winter to summer months, can range anywhere from 10^3 to 10^5 CFU per gram of feces. The most common range of *E. coli* O157:H7 being shed, during summer months, is approximately 10^3 CFU/g. However, a small proportion of cattle will shed amounts between 10^3 CFU/g and 10^5 CFU/g and this specific group is referred to as "super shedders". This group causes the most concern as they can contribute the most bacteria to carcasses and meat products (Omisakin et al., 2003).

The average test shows that *E. coli* O157:H7 prevalence, in a single herd, can be as high as 10 percent and in the overall beef cattle population the prevalence can be as high as 28 percent. The prevalence approximations may be slighter lower than actual prevalence data due to not enough herds tested, not enough animals within the herds tested, tests with low sensitivity, and seasonal variation during test times (Grauke et al., 2002). Although colonized with *E. coli* O157:H7, cattle and other ruminants show no adverse side effects from the pathogenic bacteria. The Shiga toxin receptor, needed to cause illness, has not been found in cattle and the absence presents a possible reason for the differing effects between cattle and human (Pruimboom-Brees et al., 2000).

A study conducted by Cambridge University (Hancock et al., 1994) tested the prevalence of *E. coli* O157:H7 in dairy cattle and beef cattle in Washington State. Sixty dairy herds were tested by taking samples of fecal material along with samples of bulk milk. Twenty-five beef cattle herds were tested by taking samples from the cattle hide and fecal deposits. It was concluded that 10 of the 3570 samples in dairy cattle were positive for *E. coli* O157:H7. Seven of the 10 positive samples were obtained from calves. The 10 positive samples were from 5 of

the 60 herds tested. Ten of the 1412 beef cattle fecal samples contained positive results of *E. coli* O157:H7. The 10 positive samples came from 4 of the 25 herds tested. All screening for *E. coli* O157:H7 in milk samples returned negative results. It can be suggested that beef cattle have a higher prevalence of *E. coli* O157:H7 based on the 0.7% prevalence compared to dairy cattle that retained 0.28% prevalence. Dairy cattle had *E. coli* O157:H7 in 8.3% of the herds tested, while beef cattle had the bacteria in 16% of herds tested (Hancock et al., 1994).

CHAPTER 3 - Proposed Reasons for High Prevalence of *Escherichia coli* O157:H7 in Cattle, but Low Incidence of Pathogenic Illnesses in Humans from Similar Strains.

Diversity among Conserved Genomic Regions in Human and Bovine Strains of *Escherichia Coli* O157:H7

In the many years given *E. coli* O157:H7 has been in existence, it has taken thousands of lives (CDC, 2009), caused thousands of foodborne illnesses and outbreaks (CDC, 2009), and has drawn a large amount of attention from the public, CDC and other governmental agencies such as the USDA and FDA(CDC, 2009). Pathogenic effects in the human are not apparent in the main reservoir, cattle, that serves the human population (Pruimboom-Brees et al., 2000). Not only is there a difference in the effect between human and cattle when it comes to this pathogen, but also in the prevalence of this pathogen within the human and cattle populations. There has been a low incidence of illness caused by *E. coli* O157:H7 in humans when compared to the high prevalence of *E. coli* 0157:H7 found in cattle and their environment. Research, independent studies, experiments, and analysis of clinical cases have been done in order to determine why the properties of *E. coli* O157:H7 differ greatly among humans and cattle (Besser et al., 2006, Kim et al., 1999, Pruimboom-Brees et al., 2000, Steele et al., 2007.) The genomic difference between *E. coli* O157:H7 strains have been a possible answer to why cattle prevalence and incidence of human illness has not share a linear relationship. Other possible reasons include diversity of Shiga toxin production and receptors in cattle verses human (Besser et al., 2006), the difference between the production of Locus of Enterocyte Effacement (LEE) in both human and cattle lineages (McNally et al., 2001), and the Q933 gene involvement (Ahmad and Zurek, 2006).

It has been discovered, through population genetic analysis, that *E. coli* O157:H7 and other O157:H$^-$ isolates make up a clone complex. In spite of the clonal nature of *E. coli* O157:H7 and other O157:H$^-$ isolates, significant characteristics showing variability between the clone complex has been observed through various genomic typing methods, such as pulsed field gel electrophoresis (PFGE) and octamer-based genome scanning (OBGS). These variability aspects can possibly account for the rapid divergence of *E. coli* strains including the recently discovered divergence of *E. coli* O157:H7. By the means of OBGS, it has been suggested that *E. coli* O157:H7 has diverged in to two separate lineages, lineage I and lineage II. Lineage I has been known to populate the human species whereas lineage II has appeared more in cattle.

The discovery of two separate lineages was in 1999 and has been followed by a cascade of research studies supporting its finding (Kim et al., 1999, Steele et al., 2007). Octamer Based Genome Scanning was the method used to determine the genetic diversity among human and bovine isolates of *E. coli* O157:H7. Human and bovine isolates of *E. coli* O157:H7 were found to be non-randomly distributed among the two lineages after analysis of OBGS products. The segregation of isolates has suggested that one of these lineages could be less virulent for humans or may not have the ability to be transmitted to humans by bovine sources (Kim et al., 1999). The transmission uncertainty of isolates between human and bovine species could relate to the high prevalence of *E. coli* O157:H7 in cattle, but low incidence of infection in humans comparatively.

Developed recently, was the lineage-specific polymorphism assay (LSPA-6) which is based on six loci that show bias in their allelic distribution between the two lineages. A study conducted by Steele et al. (2006) which used LSPA-6 strain and utilized the suppression subtractive hybridization (SSH) method to identify genomic regions present in *E. coli* O157:H7

lineage I strains but absent from the lineage II strains. The lineage I strains used in the study were LSPA-6 111111 and the lineage II strains were LSPA-6 222222. After identifying genomic regions of difference (RDI), (genomic sequences that were present in the lineage I strain, but absent in the lineage II strain) the study then identified conserved regions of genomic difference (CRD). These were candidates that were conserved across multiple lineage I strains and absent in multiple lineage II strains (Fig. 3.1). After the genomic regions were determined, it was noted that lineage 1 strains share a set of unique genes that are largely absent in lineage II strains. The exact contributions of these genes are unknown, but they are thought to be responsible for expression of virulence factors in lineage I strains (Steele et al., 2007).

Many studies have played a role in trying to determine the cause of the pathogenicity of *E. coli* O157:H7 and differences between humans and bovine isolate, while the findings of new lineages has been a big step.

Variety among Shiga Toxin Receptors in Human vs. Bovine Strains of *Escherichia coli* O157:H7

Shiga toxin refers to a family of toxins that is subdivided into two groups, Shiga toxin 1 (*stx*1) and Shiga toxin 2 (*stx*2). This toxin is of *Shigella dysenteriae* bacterium origin and was first described by Kiyoshi Shiga (Beutin, 2006). Two of the most common sources of Shiga toxin production has been *Shigella dysenteriae* and the Shigatoxigenic group of *Escherichia coli* (STEC). Shiga toxin is one of the main virulence factors produced in STEC.

The action of Shiga toxin has been associated with HUS and hemorrhagic colitis (HC) in multiple patients who have tested positive for *E. coli* O157:H7. Since the toxin is not recognizable to the human's immune system, and once the Shiga toxin is released from the STEC organism, the body's natural response is to increase permeability of cell barriers so

neutrophils and polymorphonuclear leukocytes (PMN) can reach the infection. PMN's are white blood cells, varying in nucleus size and shape from each other, that are responsible for attacking foreign objects in the body (American Heritage® Medical Dictionary, 2007). Using this opportunity, the toxin breaks through cell walls of the digestive tract, continuing through the Golgi apparatus to the Endoplasmic Reticulum (ER) and nuclear membrane, and then enters the blood stream and translocates to other organs such as the central nervous system and kidneys (Pritzker, 2006). This intracellular method of trafficking is referred to as retrograde transport (see Fig 3.2).

Shiga toxin is considered a 6-compenent protein structure, AB_5. The B subunit is responsible for attaching to target cell receptors, specifically globotriaosylceramide (Gb3), while the A subunit is internalized and cleaved into A1 and its other components. A1 travels to the cytoplasm where it cleaves an amino acid from the 60S ribosomal subunit in the target cell. This action prevents t-RNA binding and therefore disrupts protein synthesis. With incoherent synthesis of required proteins, functions of targeted cells seize and cell death occurs. Cell death, by apoptosis, does not occur in all cell types, but there is sufficient information suggesting that vascular lesions and tissue damage can result from apoptosis and could be the precursor to severe disease. The toxin is highly specific for the Gb3 receptor on cell surfaces in order for the B subunit to attach and the A subunit to enter the cell and for severe disease to result (Pritzker, 2006).

Certain animals, such as cattle, deer and swine do not contain this specific receptor on some cells which is a potential answer to why cattle have been a reservoir for *E. coli* O157:H7, but have not been affected by its pathogenic effects, as in humans. Bovine do however contain this receptor on their crypt intestinal epithelial cells, but escape cell death by rerouting the toxins

to lysosomes instead of the ER where they come into contact with ribosome's (Cherla et al., 2003). The bacterium is then shed through bovine feces where it can come into contact with the human population causing illnesses and outbreaks. A study performed by Pruimboom-brees et al., (2000) revealed that cattle lack the vascular receptor, Gb3, for *E. coli* O157:H7 Shiga toxins. In contrast to humans in which severe disease results from *stx* production, *E. coli* O157:H7-infected cattle are tolerant of the pathogen and remain disease free for most of their lives. On the other hand, newborn calves are affected by *E. coli* O157:H7 and suffer from fatal ileocolitis. It is unknown as to why calves are susceptible to *E. coli* O157:H7 induced disease, but are tolerant carriers of the pathogen as adults. Within the study two suggestions were made: Cattle lack receptors for *stx,* and newborn calves have receptors for *stx*, but they disappear with age. The hypothesis resulted after examination of glycolipid levels in various tissue including kidneys, ileum, rectum, brainstem and cerebrum of newborn calves. It was determined that high levels of Gb3 receptors were present in samples from the kidney, brainstem and cerebrum while there was an undetectable amount in the ileum and rectum. Undetectable amounts of Gb3 were present in all tissues from adult cattle tissue tested. The research supports the idea that adult cattle do not contain Gb3 receptors and as a result do not experience *E. coli* O157:H7 induced disease as do humans. Calves have given a new insight to Gb3 research and have provided knowledge of how receptor concentrations differ among species and age (Pruimboom-Brees et al., 2000).

Shiga toxin 2 has been found to be up to 400 times more toxic, in regards to HUS related illnesses, than Shiga toxin 1. A study was carried out in 2008 that tested for the presence of s*tx2* in various cells. Mice were given *stx2* and no *stx1*, intraperitoneally, which resulted in weight loss, paralysis and death. Examination of mice, after death, showed an increase in Ca+ flux within the cerebral, which suggested that there was an increased neurotransmitter release from

neurons caused by the toxin. Since neurons contain a high level of Gb3 receptors, it has been suggested that neurons are a main target sight for Shiga toxin and its close relative Shiga-like toxin. Gb3 is more frequently found, for unknown reasons, within renal epithelial cells, and central nervous system neurons and endothelium which is a possible factor for neurotoxicity resulting from *E. coli* O157:H7 illness (Obata et al., 2008).

A previous study conducted (Boerlin et al., 1999) was subject to determine the associations between virulence factors and STEC disease in humans. Another aim was to compare bovine and human STEC populations of major serotypes involved in human disease. A major finding in the study was discovered through multivariate analysis of five virulence factors: EHEC hemolysin (*ehxA*), a Protease (*espP*), intimin encoding gene (*eae*), *stx*1 and *stx*2 encoding genes. Multivariate analysis is a method of research that involves analysis of more than one factor at a time instead of focusing on one factor during the study. After investigation of all five virulence factors, only the *eae* and *stx*2 genes had a significant relationship. This supports the ongoing hypothesis of synergism among the adhesion intimin molecule and Shiga toxin 2 production. The second aim, using univariate analysis, determined that *eae* and *stx*2 were significantly more common in serotypes found in humans when compared to bovine strains. The opposite was found true for *stx*1. Shiga toxin 1 was more frequently isolated from serotypes not found in humans than those associated with humans (Boerlin et al., 1999). This study agrees with previous studies showing *eae* and *stx*2 are more frequent in STEC isolates causing severe disease and *stx*1 as being more frequent in some STEC isolates of bovine origin (Table 3.1). Multiple studies have studied the effects of the individual virulent factors and the specific aspects of each one. However, the study conducted by Boerlin et al. (1999) presents the idea that there is

no single factor responsible for the virulent effects produced by STEC and instead that multiple factors work together to produce the pathogenic outcome of *E. coli* O157:H7

Reported by Schmidt et al. (1995), the new genetically analyzed *ehxA*, has been associated with severe clinical disease in humans. Boerlin's previous study (1999) shows a high prevalence in *ehxA* and eae in STEC isolates regardless of disease severity. However, the strong relationship with *eae* suggests that *ehxA* is likely to assist in disease as previously suggested by Schmidt (Boerlin et al., 1999) (Fig 3.3). Shiga toxin has been capable of producing illness in humans, but not in cattle. This outcome is only partly due to the toxin being released. Besides the toxin, other virulent factors such as genetic sequences, intimin, LEE, and the Q_{933} gene all have a responsibility to the disease-causing component of *E. coli* O157:H7 and other EHEC organisms.

Even though genetic sequences, directly, are not virulent they can be the cause of virulent actions. Some virulence genes, such as *stx*1, are often regulated by iron concentration. In a study conducted in 2007, it was determined that *E. coli* O157:H7 contains a cluster of Open reading frames (ORF) within a Conserved Region of Difference in Lineage I (CRDI), specific to lineage I that may represent an iron uptake system. This could be responsible for triggering *stx*1 gene to continue on with virulent action (Steele et al., 2007).

The Role of Intimin in *Escherichia coli* O157:H7

Intimin is a vital aspect in relation to both Shiga toxin production and severe disease in humans. Intimin is an adhesion virulence factor in EHEC *E. coli* strains. It is found on the cell surfaces and is considered an attaching and effacing (A/E) protein. It can bind to its receptor, Tir, which is secreted from bacterial cells into the host cytoplasm of intestinal epithelial cells by means of Type three Secretion System (TTSS). TTSS is an organelle found mostly in Gram

negative bacteria pathogenic bacteria and is used to secret proteins to help the bacteria infect eukaryotic organisms (Salmond and Reeves, 1993).

A study performed by Cookson and Woodward (1992) tested for the role of intimin, for adherence, in *E. coli* O157:H7 strains. To carry out the study, the *eae* gene was inactivated in three strains of *E. coli* O157:H7 from various origins. Adherence of intimin to epithelial cells showed no intimate adherence, whereas wild type *E. coli* O157:H7 strains showed a high amount of adherence. Adherence in wild type *E. coli* O157:H7 was determined by findings of A/E lesions and colonies on cells. The study also looked into intimin-independent adherence using neonatal calf gut explants. The same wild type and mutant strains from the first part were used to compare adherence in colon tissue and rumen tissue in the neonatal calves. It was found that the same amount of adherence took place whether the calf was given wild type or the *eae*-inactivated version of *E. coli* O157:H7. The studies' outcome confirms that intimin does play a strong role in adherence between *E. coli* O157:H7 and various cells, but also that there could possibly be another factor contributing to bacterial adherence causing pathogenic illness (Cookson and Woodward, 2002).

Variants of the intimin molecule differ between bacterial isolates. The diversity of intimin subtypes across a diverse collection of *E. coli* O157:H7 from both bovine and human origin was examined. The basis of the study was to use Polymerase Chain Reactions (PCR) to amplify the C-terminal amino acids in the bacteria and then utilize restriction fragment length polymorphism (RFLP) analysis to differentiate the subtypes. By utilizing this method, three new subtypes of intimin were found. The origins of the new subtypes were not of importance in the study, but instead the subtype sequences. The study shows that there are undiscovered intimin

receptors and that they could be a reason for the difference between adhesion activity within pathogenic bacterial strains (Ramachandran et al., 2003).

Anti-Terminator Q933 Gene Involvement in *Escherichia coli* O157:H7 Virulence

Although ruminants appear to be the obvious reservoir for *E. coli* O157:H7, bovine serotypes are not frequently isolated from human patients. Part of the reason for this mystery could be due to the Q_{933} gene within the *E. coli* genome. The *stx*2 gene, which is regulated by the interaction between the $P_{R'}$ and Anti-Terminator Q gene, is located upstream of Anti-Terminator Q. Anti-Terminator Q initiates the late promoter, $P_{R'}$, and in doing so transcribes the *stx* gene furthering Shiga toxin production. In a study by Ahmad and Zureck (2006), it was proposed that isolates with Q_{933} produced significantly more Shiga toxin compared to isolates not containing Q_{933}. The study consisted of 262 environmental strains of *E. coli* O157:H7 which were isolated from beef cattle feces and housefly digestive tracts. All strains were screened for Q_{933} and Q_{21} (anti-terminator Q_{21} of bacteriophage 21). It was found that only 3.4% tested positive for Q_{933}, while 61.5% tested positive for Q_{21}, and 25.1% carried both Q alleles. Even though a minimal percent of the tested strains were positive for Q_{933} alone, it was these strains that had the highest Shiga toxin production. They produced higher amounts of Shiga toxin compared to strains that contained Q_{21} alone or the combination of Q alleles. These conclusions suggest that Q_{933} could be an important factor in clinically relevant strains since humans can be subjected to large amounts of Shiga toxin when infected with *E. coli* O157:H7 (Ahmad and Zureck, 2006).

It has been demonstrated that the anti-terminator Q_{933} is more common in human isolates than cattle isolates of *E. coli* O157:H7. It was also demonstrated that geographical regions that

have low *E. coli* O157:H7 illnesses also have a low number of *E. coli* O157:H7 isolates containing Q_{933} (LeJeune et al., 2004). In the study by LeJeune et al. (2004) 158 *E.coli* O157:H7 isolates were analyzed for Q_{933} and Q_{21}. Ninety-one isolates were of bovine origin and 67 were originally isolated from ill persons. Pulse Field Gel Electrophoresis and Enzyme-linked Immune Sorbent Assay (ELISA) was done on each strain to gather conclusions. It was determined that the human strains contained Q_{933} while the bovine strains contained the Q_{21} gene. Nine strains of the 158 strains initially tested were known to be of lineage I genotype and seven were of lineage II genotype. Lineage I *E. coli* O157:H7 strains are known to be more frequently isolated from humans than cattle. The study found that the lineage I strains contained the Q_{933} gene whereas the lineage II strains, which are more frequent in cattle, contained the Q_{21} gene and not the Q_{933} gene (Fig. 3.4). This could help explain why lineage I strains tend to produce Shiga toxin in higher amounts compared to lineage II strains (LeJeune et al., 2004).

The presence of Q_{933} is higher in human isolates than cattle isolates, and its presence corresponds to a higher Shiga toxin production than Q_{21}. This could relate to why cattle can carry *E. coli* O157:H7 isolates, but are asymptomatic: Cattle may simply not have *E. coli* O157:H7 isolates that contain the Q_{933} gene.

Locus of Enterocyte Effacement

The locus of enterocyte effacement (LEE) is a pathogenicity island, within the EHEC bacteria, which is clustered with genes that encode multiple virulent factors. Such genes include ones that encode intimin (*eae*), intimin's receptor (*Tir*), Shiga toxins (*stx*1 and *stx*2), EHEC hemolysin (*ehxA*), and the LEE effector molecules (*espA, espB and espD*). *Eae* and *Tir* have played a crucial role in formation of A/E lesions in several cell culture models and are expected to work simultaneously within bovine and human environments as well. *EhxA* is also known to

contribute to severe disease in humans infected with EHEC (Ritchie et al., 2003). Many studies have looked into the effects of the individual LEE encoded factors, but have not studied the combined effects or ratios of LEE factors in both human and bovine populations.

Recent epidemiological data has shown a high prevalence of *E. coli* O157:H7 in cattle and their environment, but a relatively low level of infection in humans. A common suggestion to why this has occurred promotes the pathogenic differences between human and cattle. A study performed in 2001 has tried to explain the contradicting high *E. coli* O157:H7 cattle prevalence and the low human disease incidence from *E. coli* O157:H7. A range of virulence determinants, including toxins and adhesions, were screened from human disease and bovine fecal *E. coli* O157:H7 strains. Factors were examined in relation to each other. Secreted protein profiles and actin rearrangement was investigated to note the difference between the human and bovine strains. Results showed a significant strain and medium dependent variation in *espD* and *Tir* secretion levels. The higher secretion rate of *espD* in different media suggests different ways LEE can react between different environments such as the human and bovine atmospheres. *Esp* secretion in general correlated to the amount of lesions present during examination of tissue culture. Strains of human origin produced considerably higher levels of *espD* than the majority of strains from bovine origin, which illustrated the human pathogenic potential in which bovine do not posses. The overall study has suggested that not only do human strains have increased *stx*1 and *stx*2 compared to the bovine population, but also a greater secretion and expression of other LEE-encoded factors (McNally et al., 2001).

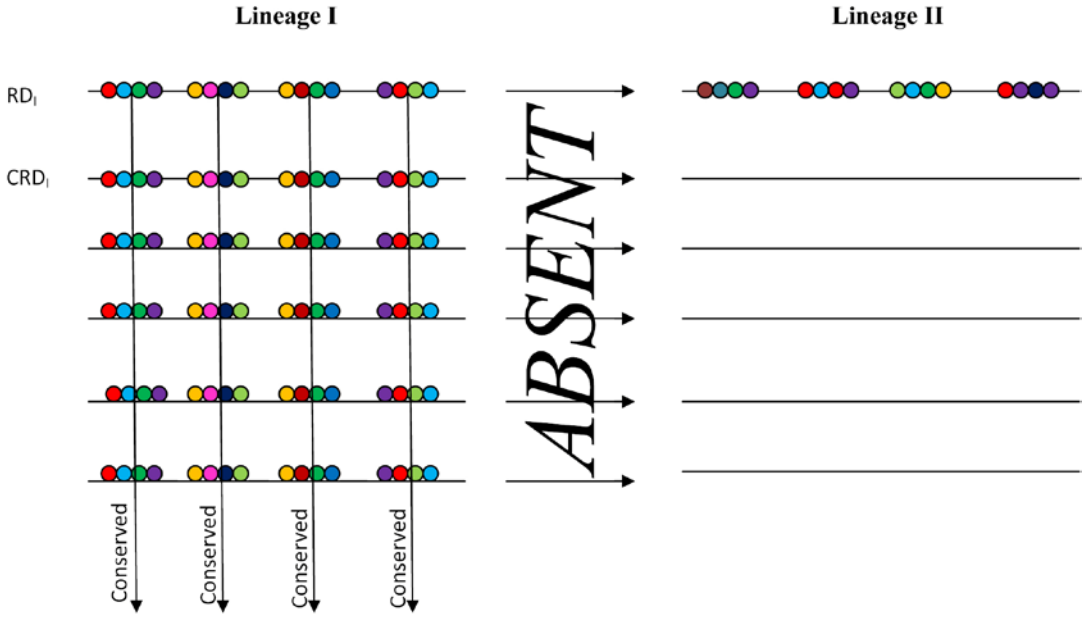
Figures and Tables

Table 3.1 Distribution of virulence factors from bovine and human STEC serotypes. The numbers in the columns represent the number of isolates tested for the specified gene. The number in parentheses represent of the total category positive for that specific gene. The serotypes were analyzed for 5 major genes in both bovine and human isolates. Serotypes used were: O26:H11, O103:H2, O111:H8, O111:H$^-$, O145:H$^-$, and O157:H7

Gene	Total	Bovine	Human	Severe disease
ehxA	146 (61.0)	59 (60.2)	87 (62.6)	64 (71.9)
espP	148 (62.4)	60 (61.2)	88 (63.3)	55 (61.8)
Eae	77 (32.5)	23 (23.5)	54 (38.8)	43 (48.3)
stx1	148 (62.4)	72 (73.5)	76 (54.7)	46 (53.9)
stx2	134 (56.5)	42 (42.9)	92 (66.2)	63 (70.8)
No. of isolates	237	98	139	89
No. of serotypes	118	53	65	39

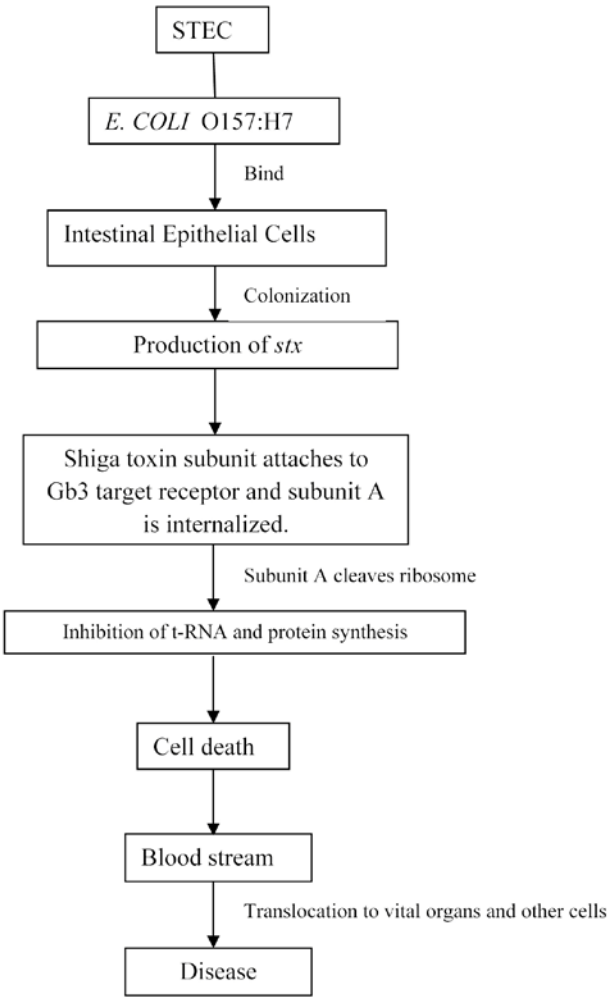
(Boerlin *et al.*, 1999)

Figure 3.1 Depiction of conserved genetic regions through multiple lineage I strains and their absence in lineage II strains of *Escherichia coli* O157:H7.



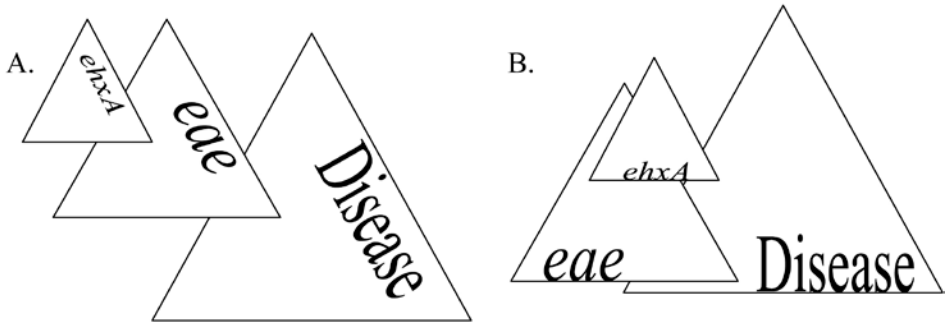
(Page, 2009)

Figure 3.2 Model of disease pathway concerning *Escherichia coli* O157:H7 and Shiga-toxin.



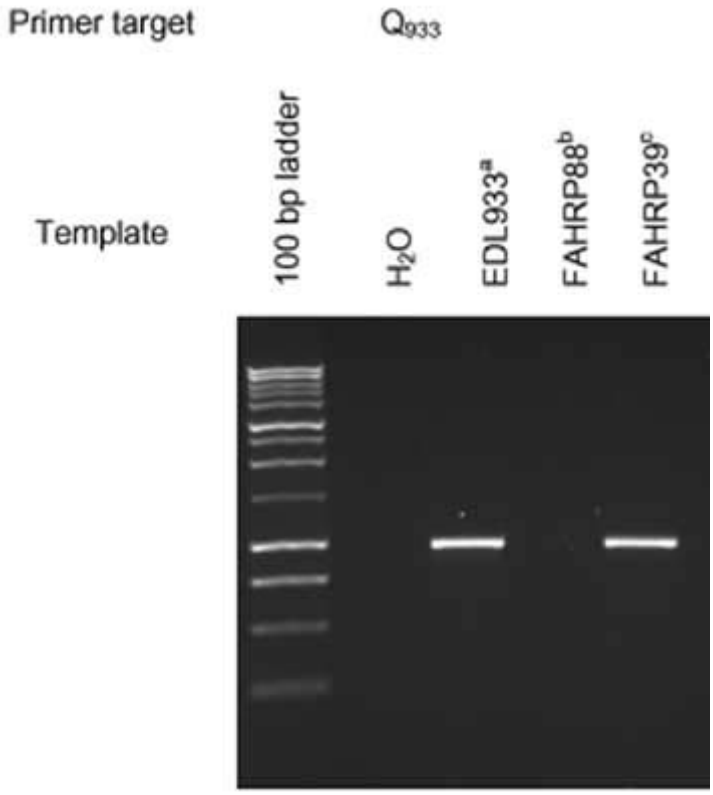
(Pritzker, 2006)

Figure 3.3 (A) Diagram representing the indirect relationship between the EHEC hemolysin molecule and disease in humans. (B) Diagram representing the direct relationship between the EHEC hemolysin molecule and disease occurrence in humans.



(Boerlin et al., 1999)

Figure 3.4 PCR products of human verses bovine strains of *Escherichia. coli* O157:H7 and whether or not they contain Q_{933} gene. EDL933 (human-origin) shows Q_{933} gene. FAHRP88 (bovine-origin) does not contain the Q_{933} gene).



(LeJeune et al., 2004)

CHAPTER 4 - The Effects of *Escherichia coli* O157:H7 on the human population: Case Studies

A multistate outbreak of *E. coli* O157:H7 during December and November of 2000 led the USDA FSIS to announce a class 1 recall on ground beef. A Green Bay, Wisconsin meat plant voluntarily recalled 1.1 million pounds of ground beef that had been distributed to 15 different states. The Wisconsin Division of Public Health (WDPH), along with local health departments, and the Wisconsin State laboratory of Hygiene (WSLH) conducted an in-depth investigation which involved reported *E. coli* O157:H7 cases, isolated strains of *E. coli* O157:H7 and interviews to all suspected cases of *E. coli* O157:H7. In a press release from the WDPH, symptoms and sign of infection were made clear to the public and symptomatic persons were urged to attend their local health department for medical attention and submission of a stool sample to identify possible *E. coli* strains. Possible *E. coli* O157:H7 infections were reported to the WDPH from local health agencies while all *E. coli* O157:H7 isolates from specimen were forwarded to the WSLH for further confirmation using standard methods recommended by the Centers of Disease Control and Prevention (CDC). Pulse field gel electrophoresis (PFGE) along with traditional agar methods were utilized to receive the most accurate results. During the investigation, 74 laboratory- confirmed cases of *E. coli* O157:H7 were reported from the WDPH. The patient age ranged from 3 to 84 including six hospitalizations and one case of HUS. After further investigation of *E. coli* O157:H7 isolates, it was concluded that there was a total of four different patient strains of *E. coli* O157:H7 involved in the outbreak (Proctor et al., 2002). This study gives us insight as to how many various strains can reside within cattle populations compared to the number of strains that cause disease in humans. The difference in strains could

explain the difference in symptoms among people and the severity of diseases ranging from diarrhea to HUS.

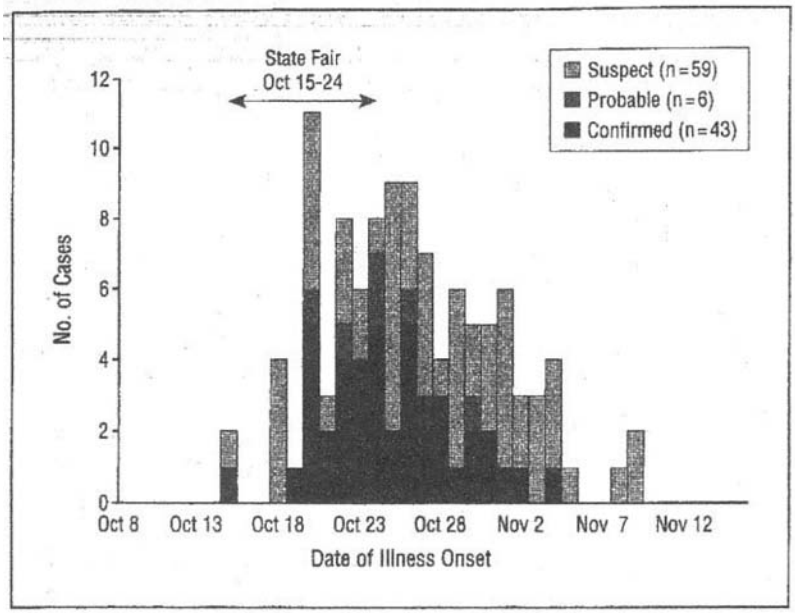
Escherichia coli O157:H7 has not only been passed from cattle to human by means of food products. Physical contact between human and cattle and their environment has been a substantial risk of *E. coli* infection if proper measures regarding safety and cleanliness were not considered. In October 2004 one of the largest petting zoo outbreaks of *E. coli* O157:H7 occurred during the North Carolina State Fair. After three HUS infections were reported in the state, the North Carolina State Laboratory for Public Health and the US department of Agriculture conducted an investigation to find the source of the Shiga-toxin-induced HUS infections. 96 initial environmental samples were taken from fair grounds including: animal bedding, manure from animal sites, swabs from floors and fans, swabs from apple cider presses, water samples from fountains, and live flies in animal exhibits. Between October 8 and November 12, 2004 there were 108 outbreak related cases. 43 of these cases were laboratory-confirmed *E. coli* O157:H7 infections and overall 15 cases of HUS were reported (Fig. 4.1). The median age was 5.4 years suggesting that mouth-to-hand activities, such as thumb sucking, and eye rubbing could have been the means of bacterial transfer between the state fair and humans. The environmental sampling did not support hypothesis of food or water borne transmission. It was concluded that 1 of the 2 petting zoo locations within the state fair was consistently contaminated with *E. coli* O157:H7. This location was of popularity as it was an area where visitors could directly interact with approximately 100 sheep and goats. Some live stock exhibits had slight STEC O157 contamination, but was thought to spread from the same petting zoo location that possessed high numbers of *E. coli* O157:H7 when investigated (Goode et al., 2009). This case study shows how education is important for protection against bacterial transmission.

Products such as hand sanitizers have increased awareness and healthy habits, but is not always utilized which can produce a cascade of person to person transmission.

In 1993, a large *E. coli* O157:H7 outbreak occurred in California due to undercooked meat and hamburger patties distributed by the national food chain, Jack-in-the-Box. People became sick in multiple states, from multiple locations of this chain, including Seattle, Idaho and Nevada. Overall, four children died due to HUS and over 600 became ill. After investigation it was discovered that the ground beef patties were actually contaminated with fecal material causing the severe illnesses in people. It was known as the largest *E. coli* O157:H7 outbreak in America up to that time (Department of Defense, 1993). This shows how improper safety measures can effect food products and put millions of people at risk. Since then, numerous safety precautions have been made, such as HACCP and GMP's to help prevent foodborne illnesses and hinder bacteria from transmission.

Figures and Tables

Figure 4.1 Dates of illness onset due to Shiga-toxin producing *E. coli* outbreak, from North Carolina State Fair, in 2004.



(Goode et al., 2009)

CHAPTER 5 - The Importance of High *Escherichia coli* O157:H7 Prevalence in Cattle in Regards to the Relatively low Incidence of *Escherichia coli* O157:H7-Induced Disease in Humans

Multiple studies have supported the idea that *E. coli* O157:H7 colonizes in high prevalence within wildlife animals with cattle being the main reservoir. On the other hand, incidences of disease in humans remain relatively low even with the low infectious dose of the organism. It is important to understand this relationship to help monitor what strains are capable of transmission between cattle and human populations. Different *E. coli* O157:H7 isolates contain different pathogenic potentials and thus are not always capable of transfer between species. If the prevalence of various strains is known, then being able to monitor those strains in humans could give insight to the simplicity of transmission for specific isolates.

There are major differences among Shiga-toxin production, virulent factors, LEE and genetic sequencing regarding strains of *E. coli* that effect humans yet do not promote effects in cattle. This supports the findings of Kim et al. (1999) proposing two different lineages of *E. coli* O157:H7. Perhaps the prevalence in cattle includes many isolates of *E. coli* O157:H7, while only a few of those are capable of producing pathogenic effects in humans due to the numerous pathogenic differences among strains.

CHAPTER 6 - Possible Areas of Future Research

Genetic analysis of *E. coli* O157:H7 and its lineages could potentially give more answers to why the bacteria is so prevalent in cattle yet has such little incidence of infection in human, comparatively. It was determined that iron uptake could trigger *stx*1. If this is the case, then perhaps research concerning bovine and human iron intake could become another area of interest. *E. coli* O157:H7 isolates in humans contain less *stx*1 than bovine strains, so maybe bovine have a greater influx of iron in general, possibly through feed. Iron uptake could also have an inverse relationship with *stx*2. Humans acquire higher amounts of *stx*2 and less *stx*1, suggesting humans do not have the required iron intake to activate the *stx*1 the same way as the bovine population. The possible lower concentration of iron in humans could, in return, account for the increased activation of *stx*2 that bovine do not have.

Another area of interest for possible research could be Shiga toxin presence in old vs. young humans. In Pruimboom-Brees study (2000) it was determined that calves had receptors as do humans whereas adult cattle contained an undetectable amount of receptors. Calves vs. cattle could be a new insight to age being a factor of *E. coli* O157:H7-induced disease. Determined, through multiple studies, HUS is caused by Shiga toxin production and is most common in small children. Receptor studies in children verses the elder could be an area of growth in research.

Growth in the area of research involving virulence factors should continue as only some questions have been answered and more questions are arising. Linkages between the multiple factors and correlating them to disease and various strains, as in bovine and human, can eliminate bias in future studies. Focus on the effects of the virulent factor combinations instead of the effects of each separate factor may give more insight to how bovine strains are different than human strains of *E. coli* O157:H7. For example, the study done by Boerlin et al. (1999)

confirmed that eae and *stx2* did not only have a strong correlation to each other, but also possessed a higher frequency in human serotypes than in non-human serotypes and a higher frequency in diseased humans compared to non-diseased humans. It can be purposed that the reaction between the two genes promotes a type of cascade reaction within the immune response system resulting in disease effects, such as HUS and HC. Since bovine strains do not have the Gb3 receptor that is vital for *stx2* to function, then perhaps reactions with *eae* are not possible and therefore omitting the pathogenic affects that are apparent in humans.

Intimin is vital in producing Shiga toxin and disease in humans. Perhaps further research in intimin subtypes verses *E. coli* serotypes could give clues as to why *E. coli* O157:H7 is more likely to produce disease when compared to other O157:H^- isolates. Another benefit to intimin research would be the ability to characterize *E. coli* strain serotypes based on the subtype of intimin it possesses. One last aspect to research in this field is the ability to correlate initmin subtypes found in bovine verses initmin subtypes found in humans. This could be a potential factor in why cattle do not adhere Shiga toxin to the ER, but instead push it to lysosomes, which makes them asymptomatic to *E. coli* O157:H7 disease.

Locus of Enterocyte Effacement is largely responsible for encoding virulent factors that produce disease. Future research regarding LEE effector molecules could potentially explain the difference of virulence between cattle and human reactions to *E. coli* O157:H7 presence. *EspD* is significantly higher in humans along with *stx*2 and *eae*. A combination of these three could explain the difference in pathogenic effects between cattle and human. LEE houses critical factors involved in *E. coli* O157:H7-induced disease and could be the potential target for pathogenic differences between cattle and human populations. Factors that affect LEE induction

and the gene-encoding activity could be of interest as well since disease begins at the pathogenic island.

Attention to more specific biotechnology could help distinguish *E. coli* O157:H7 strains from one another. This in return could help increase monitoring and surveillance of different STEC strains as they transfer from cattle to human. Knowledge of strains' capabilities, in terms of transmission, could lead to better understanding of the pathogenic qualities that differ from serotype to serotype.

REFERENCES

American Heritage® Medical Dictionary. 2007. The American Heritage Medical Dictionary, 3 ed. Houghton Mifflin Company., Boston, MA.

Ahmad, A. and L. Zurek. 2006. Evaluation of the Anti-Terminator Q933 Gene as a marker for *Escherichia coli* O157:H7 with high Shiga Toxin Production. Current Microbiology 53:324-328.

Besser, R. E., P. M. Griffin, and L. Slutsker. 1999. *Escherichia coli* O157:H7 Gasteroenteritis and the Hemolytic Uremic Syndrome: An Emerging Disease. Annual Review of Medine 50:355-367.

Beutin, L. 2006. Emerging enterohaemorrhagic *Escherichia coli*, causes and effects of the rise of a human pathogen. Journal of Veterinary Medicine, Infectious Diseases and Veterinary Public Health 53:299-305.

Beutin, L., A. Miko, G. Krause, K. Pries, S. Haby, K. Steege and N. Albrecht. 2007. Identification of Human-Pathogenic Strains of Shiga Toxin-Producing *Escherichia coli* from Food by a Combination of Serotyping and Molecular Typing of Shiga Toxin Genes. Applied and Environmental Microbiology 73:4769-4775.

Boerlin, P., S. McEween, F. Boerlin-Petzold, J. Wilson, R. Johnson and C. Gyles. 1999. Association between Virulence Factors of Shiga Toxin-producing *Escherichia coli* and Disease in Humans. Journal of Clinical Microbiology 37:497-503.

Byres, E., A. Paton, J. Paton, J. Lofling, D. Smith, M. Wilce, U. Talbot, D. Chong, H. Yu, S. Huang, X. Chen, N. Varki, A. Varki, J. Rossjohn and T. Beddoe. 2008. Crystal structure of the B-subunit of the AB5 toxin from *E. coli*. Protein Data Bank: 3DWA.

California Food Emergency Response Team. 2007. Investigation of an *Escherichia coli* O157:H7 Outbreak Associated with Dole Pre-Packaged Spinach. California Department of Health Services Food and Drug Branch. 1-50.

Carter, A., A. Borcayk, J. Carlson, B. Harvey and J. K. Hockin. 1987. A severe outbreak of *Escherichia coli* O157:H7- associated hemorrhagic colitis in a nursing home. New England Journal of Medicine 316:1496-1500.

Centers for Disease Control and Prevention. 2009. *Escherichia coli*: Previous outbreaks. National Center for Zoonotic, Vector-Borne, and enteric Diseases. <http://www.cdc.gov/ecoli/>

Center for Science in the Public Interest. 2009. Outlook Alert database, *E. coli* O157:H7. Online database.

<http://www.cspinet.org/foodsafety/outbreak/outbreaks.php?column=pathogen&colval=E.%20coli%20O157:H7>

Cherla, R., S. Lee and V. Tesh. 2003. Shiga toxins and apoptosis. FEMS Microbiology Letters 228:159-166.

Cimolai, N., B. Morrison and J. Carter. 1992. Risk factors for the central nervous system menifestations of gasteroenteritis-associated hemolytic-uremic syndrome. Pediatrics 90:616-621.

Clark, M. 2005. Jack-in-the-Box *Escherichia coli* Outbreak, About *Escherichia coli*. Online Post: 2009. Marler Clark LLP, PS, Seattle, Washington.

Cookson, A. and M. Woodward. 2002. The role of intimin in the adherence of enterohaemorrhagic *Escherichia coli* (EHEC) O157 : H7 to HEp-2 tissue culture cells and to bovine gut explant tissues. International Journal of Medical Microbiology 292:547-553.

Department of Defense. 1993. Case Study: Jack-in-the-Box *E. coli* crisis. Department of Defense. United States of America.

Gavin, P., L. Peterson, A. Pasquariello, J. Blackburn, M. Hamming, K. Kuo, and R. Thomson. 2004. Evaluation of Performance and Potential Clinical Impact of ProSpecT Shiga Toxin-Producing *E. coli* in Stool Samples. Journal of Clinical Microbiology 42:1652-1656.

Goode, B., C. O'Reilly, J. Dunn, K. Fullerton, S. Smith, G. J. Keen, L. Durso, M. Davies and S. Montgomery. 2009. Outbreak of *Escherichia coli* O157. Archives of Pediatrics & Adolescents Medicine 163:42-47.

Grauke, L. J., Kudva, I. Yoon, J. Hunt, C. Williamsand C. Hovde. 2002. Gastrointestinal Tract Location of *Escherichia coli*. Applied and Environmental microbiology.68:2269–2277.

Hancock, D., T. Besser, M. Kinsel, P. Tarr, D. Rice, and M. Paros. 1994. The Prevalence of *Escherichia coli* O157:H7 in dairy and beef cattle in Washington State. Epidemiology and Infection 113:199-207.

Kaper, J. and A. O'Brien. 1998. *Escherichia coli* O157:H7 and other Shiga toxin-producing *E. coli* strains. American Society for Microbiology. Washington, DC. 1-11.

Kim, J., J. Nietfeldt and K. A. Benson. 1999. Octomer-based genome scanning distinguishes a unique subpopulation of *Escherichia coli* O157:H7 strains in cattle. Proceedings of the National Academy of Sciences 96:13288-13293.

LeJeune, J., Abedon, S., Takemura, K., Christie, N., Sreevastan, S. 2004. Human Escherichia coli O157:H7 Genetic Marker in Isolates of Bovine Origin. Emerging Infectious Diseases. 10: 1482-1485.

McNally, A., A. Roe, S. Simpson, F. Thomson-Carter, E. Hoey, C. Currie, T. Chakraborty, D. Smith, and D. Gally. 2001. Differences in Levels of secreted Locus of Enterocyte Effacement Proteins between Human Disease-Associated and Bovine *Escherichia coli* O157. Infection and Immunity 69:5107-5114.

Merritt, E. and W. Hol. 1995. AB_5 Toxins. Current Opinion in Structural Biology 5:165-171.

Obata, F., K. Tohyama, A. Bonev, G. Kolling, T. Keepers, Gross, LK., Nelson, MT., S. Sato, and T. Obrig. 2008. Shiga toxin 2 affects the central nervous system through receptor globotriaosylceramide localized to neurons. Journal of Infectious Dieseases 198:1398-1406.

Omisakin, F., M. MacRae, I. D. Ogden, and N. J. C. Strachan. 2003. Concentration and prevalence of *Escherichia coli* O157 in cattle feces at slaughter. Applied Environmental Microbiology 69:2444-2447.

Ostruff, S. M., D. P. Hopkins, E. G. Sowers and N. K. Strockhine. 1991. Surveillance of *Escherichia coli* O157 isolation and confirmation. MMWR CDC Surveillance Summary 40:1-6.

Page, J. 2009. Personal works. Master Program. Kansas State University.

Paton, A., Beddoe, T., Thorpe, C., Whisstock, J., Wilce, M., Rossjohn, J., Talbot, U. and Paton, J. 2006. Crystal Structure of the A-subunit of the AB5 Toxin from *E. coli*. Protein Data Bank: 2IY9. <http://www.rcsb.org/pdb/explore/explore.do?structureId=2IY9>

Pollan, M. 2006. The Vegetable-Industrial Complex, The New York Times. The New York Times, New York City.

Pritzker, F. 2006. Shiga Toxin. Pritzker Olsen Attorneys. <http://www.rcsb.org/pdb/explore/explore.do?structureId=2IY9>

Proctor, M., T. Kurzynski, C. Koschmann, J. Archer and J. Davis. 2002. Four strains of *Escherichia coli* O157:H7 Isolated from Patients during an Outbreak of Disease Associated with Ground Beef: Importance of Evaluating Multiple Colonies from an Outbreak-Associated Product. Journal of Clinical Microbiology 40:1530-1533.

Pruimboom-Brees, I., T. Morgan, M. Ackermann, E. Nystrom, J. Samual, N. Cornick and H. Moon. 2000. Cattle lack vascular receptors for *Escherichia coli* O157:H7 Shiga toxins. Proceedings of the National Academy of Sciences 97:10325-10329.

Ramachandran, V., K. Brett, M. Hornitzky, M. Dowton, K. Bettelheim, M. Walker and S. Djordjevic. 2003. Distribution of Intimin Subtypes among *Escherichia coli* Isolates Ruminant and Human Sources. Journal of Clinical Microbiology 41:5022-5032.

Riley, L., R. Remis, S. Helgerson, H. McGee, J. Wells and B. Davis. 1993. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. New England Journal of Medicine 308:681-685.

Ritchie, J., C. Thorpe, A. Rogers, and M. Waldor. 2003. Critical Roles for *stx2*, eae, and tir in Enterohemorrhagic *Escherichia coli*-induced Diarrhea and Intestinal Inflammation in Infant Rabbits. Infection and Immunity 71:7129-7139.

Robson, W., A. Leung and M, Montgomery. 1991. Causes of death in hemolytic uremic syndrome. Child Nephrology & Urology 11:228-233.

Salmond, P. and G. Reeves. 1993. Membrane traffic wardens and protein secretion in Gram-negative bacteria. Trends in Biochemical Science 18:17-20.

Schmidt, H., Beutin, L., Karch, H. 1995. Molecular Analysis of the Plasmid-Encoded Hemolysin of *Escherichia coli* O157:H7 strain EDL 933. Infection and Immunity. 63: 1055-1061.

Steele, M., K. Ziebell, Z. Yongxiang, A. Benson, P. Konczy, R. Johnson and V. Gannon. 2007. Indentification of *Escherichia coli* O157:H7 Genomic Regions Conserved in Strains with a Genotype Associated with Human Infection. Applied and Environmental Microbiology 73:22-31.

Su, C. and L. Brandt. 1995. *Escherichia coli* O157:H7 Infection in Humans. Annals of Internal Medicine 123:698-714.

Tarr, P. 1995. *Escherichia coli* O157:H7: Clinical, Diagnostic, and Epidemiological Aspects of Human Infection. Clinical Infectious Diseases 20:1-10.

Tarr, P., M. Neill, J. Allen, C. Siccardi, S. Watkins and R. Hickman. 1989. The increasing incidence of the hemolytic-uremic syndrome in King County, Washington. American Journal of Epidemiology 129:582-586.

United States Department of Agriculture Food Safety and Inspection Service. 2006. Food Safety Education: Is it done yet? <http://www.fsis.usda.gov/Is_It_Done_Yet/Brochure_Text/index.asp>

Wachsmuth, I. K., P. H. Sparling and M. E. Potter. 1997. Enterohemorrhagic *Escherichia coli* in the United States. FEMS Immunology and Medical Microbiology 18:233-239.