Review On Shiga Toxin Escherichia Coli In Milk And Their Public Impacts

Jimma, Ethiopia

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Abstract: Milk is an important source of nutrients to human and animals, as it is considered as best, ideal and complete food for all age groups. However, in spite of being so, milk can also serve as a potential vehicle for transmission of some disease under certain circumstances. Because of their unique composition and properties, this is excellent growth media for many pathogenic microorganisms. Milk borne transmission of Shiga Toxin producing *Escherichia coli* has raised considerable concern due to recent outbreaks worldwide and poses a threat to public health. As ruminant are health carriers of STEC and most dairy products may provide these bacteria with favorable conditions for their growth, milk and dairy products are a potential source of STEC. Among those *Escherichia coli* O157:H7 are the most frequent potential pathogens associated with milk or dairy products in many countries and are therefore the main microbiological hazards linked to dairy products. *Escherichia coli* are versatile species encompassing both commensals of the digestive tracts of many vertebrates, including humans, and pathogenic strains causing various intra and extra intestinal infections. Pathogenic strains of this organism are distinguished from normal flora by their possession of virulence factors such as exotoxins. *Escherichia coli* O157:H7 is associated with life threatening diseases such as hemorrhagic colitis, hemolytic uremic syndrome and thrombotic thrombocytopenic purpura. This review summarizes the scientific information about Shiga Toxin producing *Escherichia coli* related to foodborne pathogens in dairy products and highlights the role of milk for the transmission and associated public health impacts.

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1. Introduction

Farm animals represent a major reservoir of pathogens that can be transferred to milk (Arqués *et al*., 2015). Milk is an important source of nutrients to human and animals. It is meant to be the first and the only food for the offspring of mammals as is almost complete food (Pandey and Voskuil, 2011). Being rich in proteins, lipids and sugars, milk is an example of ideal culture medium for various microorganisms. Some of the bacteria contained in milk like Lactobacillus species Bifidobacterium are also present in the healthy human gastrointestinal tract, aiding in digestion and protection from other infections, while other bacteria can be extremely harmful to human health (Baffoni *et al*., 2012). In natural conditions, the microbial composition of milk is influenced by different parameters, such as the microorganisms present in the teat canal, on the surface of teat skin, or in the surrounding air, as well as the animal's feed, the quality of the water supply, and equipment hygiene (Quigley *et al*., 2013).

Food-borne diseases are important public health and economic burden (Schlundt *et al*., 2004). Milk is considered a high risk food as it is highly nutritious and serves as an ideal medium for bacterial growth (Chye *et al*., 2004). Additionally, foodborne bacteria can contaminate food products at any point along the production chain, milking, storage or packaging (Tomat *et al*., 2016).

Several outbreaks have been associated with the consumption of dairy products, particularly milk/cheese and other ready to eat foods (Melo *et al*., 2015). *Escherichia coli* O157:H7, *S. aureus*, *L. monocytogenes* and others are the most frequent potential pathogens associated with milk or dairy products in many countries (Jakobsen *et al*., 2011) and are therefore the main microbiological hazards linked to raw milk and milk products. Finally, the main reservoirs of Shiga toxin-producing Escherichia coli are ruminants, contaminating milk through subclinical mastitis or feces, and the bacteria can persist in milking equipment (Arqués *et al*., 2015).

Escherichia coli, a member of the bacterial family of Enterobacteriaceae, are the most prevalent commensal inhabitant of the gastrointestinal tracts of humans and warm-blooded animals, as well as one of the most important pathogens. As a commensal it lives in a mutually beneficial association with hosts, and rarely causes diseases (Kaper, *et al*., 2004). The development of a disease after consumption of contaminated dairy products made from raw milk depends on several factors, such as the pathogenicity

of the bacteria strain, the number of ingested microorganisms, the physiological state of the microorganism, and the health condition of the consumer at the moment of ingestion (Verraes *et al*., 2015).

Dairy farms act as reservoirs for several food-borne pathogens such as Shiga-Toxin producing Escherichia coli, (Oliver *et al*., 2005, Vimont *et al*., 2007) and the major source of STEC strains in milk that contaminate milk and meat through direct contact with the cattle and the dairy farm environments (Hussein and Sakuma, 2005). It has been reported that the transmission can occur through the contaminated milk and milk products (Gillespie *et al*., 2003; Chye *et al.,* 2004). Raw milk exposed to untreated and contaminated water, cattle or human faeces can easily be contaminated with E. coli. Unpasteurized milk and dairy products made from raw milk act as vehicles for transition of E. coli to human (Dweik *et al*., 2012). Raw milk is known as the main transmission pathway for pathogens resulting in food-borne outbreaks every year (CDC, 1999; Hussein and Sakuma, 2005). *E. coli* O157:H7 was first recognized as a foodborne pathogen in 1982 during an investigation into an outbreak of hemorrhagic colitis (bloody diarrhea) associated with consumption of contaminated hamburgers (Riley, *et al*., 1983). Some strains like O157 and other EHEC cause no discernible disease in their animal reservoirs; however, diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome are not uncommon in humans (Garcia *et al*., 2010).

Milk production in Ethiopia is largely from the smallholder farmers in the highlands and the pastoralists in low land areas of the country. However, the production is not market oriented and a minor portion of the locally produced milk enters the commercial sector owing to the marketing constraints and lack of processing techniques suitable for smallholder dairying (Kelay, 2002). However, several pathogenic *E. coli* strains have emerged that cause disease in humans from dairy products. Pathogenic *E. coli* can be divided into intestinal pathogens causing diarrhea, and extra intestinal E. coli causing a variety of infections in both humans and animals (Lucia *et al.,* 2015). *E. coli* found in humans can be categorized on basis of genetic and clinical criteria into three main groups: commensal, pathogenic (enteric or diarrheagenic) and extraintestinal pathogenic E. coli (ExPEC).

It is not known just when milk was first suspected as being an agent for the transmission of disease. Worldwide, virulent strains of E. coli are emerging, as they have the potential to cause food borne illness (Rangel *et al*., 2005). Shiga toxin-producing Escherichia coli are emerging food borne zoonotic pathogens associated with hemorrhagic colitis and HUS in humans (Abdallah *et al*., 2014). As Haluk, (2008) stated that HUS is the most worrisome complication of EHEC infections and is characterized by the triad of acute renal failure, and thrombocytopenia, with a fatality rate between 2% and 7%. As Ferreira *et al* (2014) reported that, the high prevalence of STEC in dairy cattle poses a significant risk to public health, since these microorganisms can contaminate products intended for human consumption, like milk, water, meat products, dairy products, and/or products of plant origin. As for other zoonotic agents, having animals and raw products that are free from STEC is not possible in practice (Fairbother and Nadaeu, 2006). As Alfredo *et al* (2004) stated that their occurrence can be minimized by applying high standards of hygiene in all the steps of the food production chain. The review approaches an understanding will allow the development of effective strategies to eliminate and reduce the numbers of the Shiga toxin producing *Escherichia coli* in the animal reservoir, source and ways of circulating in the environment, problems of contaminated dairy products and public health implication. So far there was any review and well documented review regarding shiga toxin E.coli in milk and their public impacts at College of Agriculture and Veterinary Medicine, Jimma University. Therefore this review was made with the following general and specific objectives.

1.1. General Objectives

This review aimed at assessing the relation of common dairy products (milk) and determines the occurrence of producing STEC disease. Also the overview of relevant evidence likely to be associated with public health impacts of *Escherichia coli* from milk sources.

1.2. **Specific objectives**

- ✓ To assess the role of dairy products for occurrence of *Escherichia coli*.
- ✓ To observe the possible risk factors for STEC contaminations of animal products and
- ✓ To highlight pathogenic nature of *Escherichia coli* in milk and public health impacts.

2. Literature Review

2.1. Milk Borne Infections and Pathogens

Various bacteria may have access to milk and milk products from different sources and cause different types of milk-borne illnesses. Sometimes milk and milk products may carry microorganisms or their toxic metabolites (poisons/toxins). Some of these microorganisms are pathogenic and cause illness to humans while others cause spoilage in milk rendering it unsuitable for human consumption (Parekh and Subhash, 2008, Bukuku, 2013). Many milk borne epidemics of human diseases are spread through

consumption of contaminated milk (Parekh and Subhash, 2008). Few examples of the known milk borne diseases are *E. coli* O157:H7, BTB, campylobacteriosis and others as emerged new milk borne bacterial pathogen reported recently with a very serious health effects. These are zoonotic disease diseases which are transmitted to consumers and pose a risk to public health. To protect consumers and public health against these milk borne infections it require proper hygienic milking and milk handling procedures (Fairbrother and Nadaeu, 2006). *E. coli* are potential food poisoning pathogens which are widely distributed in low numbers in food environments and the most common contaminant of raw and processed milk (Quinn *et al*., 2002; Fairbrother and Nadaeu, 2006).

2.2. Characteristics of *Escherichia coli*

The bacterium *Escherichia coli*, originally known as Bacterium coli commune, and were first isolated and characterized in 1885 by the German scientist and pediatrician Theodore Escherich. E.coli is gram-negative, rod-shaped bacteria and is belongs to the family of enterobacteriaceae, the bacteria naturally and harmlessly exist in the intestines of all warm-blooded animals (Fairbrother and Nadaeu, 2006). Other species of the genus *Escherichia* include *E. adecarboxylata*, *E. blattae, E. fergusonii, E. hermanii* and *E. vulneris* (Meng and Schroeder, 2007). Certain isolates of Escherichia coli have been implicated in a wide range of diseases that affect either animals or humans worldwide (Mora *et al*., 2011).

2.3. Classification of Escherichia coli

2.3.1. Escherichia coli O157:H7

Escherichia coli that live in the human intestine cause no diseases. However, one EHEC serotype, O157, which often resides in cattle, releases toxins that can cause severe illness in humans, and only a small number (fewer than 10 bacteria) are required to cause serious human illness (Paton *et al*., 1996; Fairbrother and Nadaeu, 2006). Pathogenic *E. coli* are classified into specific groups based on the mechanisms by which they cause disease and clinical symptoms. These categories include EHEC, EAEC, EIEC, EPEC, ETEC and DAEC (Montville and Matthews, 2005).

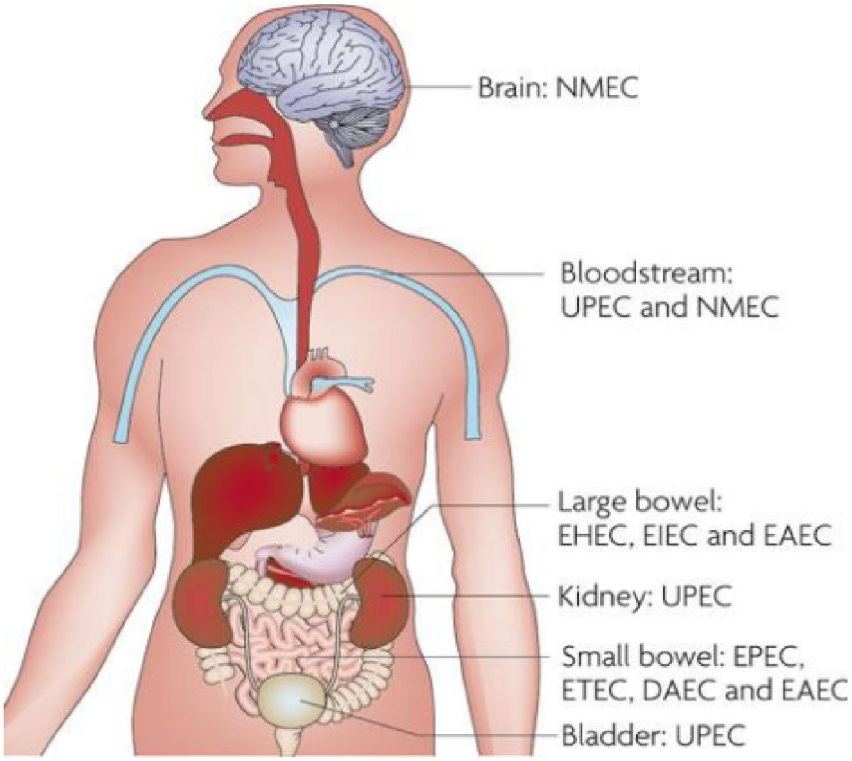

Figure1. Sites of pathogenesis *E. coli* colonization in humans
(Source: Croxen and Finlay, 2010)

2.3.2. Shiga toxin producing Escherichia coli (STEC)

Shiga Toxin *Escherichia coli* are Shiga-toxin producing *E. coli*, also known as VTEC. The STEC

strains that cause haemorrhagic colitis (bloody diarrhoea) belong to the EHEC group of pathogenic *E. coli* (Yoon and Hovde, 2008). In developed countries EHEC is the most serious of the pathogenic *E. coli*, however, in developing countries EPEC is a major disease causing agent in children (Meng and Schroeder 2007; Ochoa *et al*., 2008). Strains of *E. coli* can be characterized serologically based on the detection of specific O, H and K antigens. For most E. coli strains the O and H antigens are sufficient to identify the strain. For example, *E. coli* O157:H7 is the leading cause of STEC infections internationally (Gyles, 2007; Meng and Schroeder, 2007).

Diarrheagenic *E. coli* strains are among the most common etiologic agents of diarrhea and based on their specific virulence factors and phenotypic traits are divided into EPEC an important cause of infant diarrhea, ETEC a major cause of travelers' diarrhea and infant diarrhea in less developed countries, Vero toxin-producing/Shiga toxin-producing *E. coli* include its well-known subgroup EHEC a cause of hemorrhagic colitis and hemolytic uremic syndrome, EIEC a cause of bacillary dysentery, EAEC, and DAEC (Montville and Matthews, 2005; Karmal *et al*., 2010). STEC bacterium is one of the major bacterial pathogens causing food-borne illnesses, ranging from mild diarrhea to a life threatening complication known as HUS (Friedrich *et al*., 2002).

As the capacity to induce illness depends on virulence factors that are required to assess the public health significance of emerging non-O157 strains. Non-O157 Shiga toxin-producing Escherichia coli (STEC) strains have been linked to outbreaks and sporadic cases of illness worldwide (Mathusa *et al.,* 2010). Non-O157 STEC has been found in the animal population worldwide, including in Africa and the People's Republic of China (Fairbother and Nadeau, 2006). Non-O157 STEC is mostly associated with cattle but has also been isolated from sheep, goats, pigs, and chickens.

Table 1: Classification of Shiga toxin producing *Escherichia coli* in found in animals

Type	STEC subsets: common designation	Common serotypes/groups	Geographical distribution	Animal reservoir	Site of isolation in animals and derived products
Zoonotic	O157 EHEC	O157:H7	Worldwide, industrialised countries	Cattle, shoats, *pigs*	milk, cheese, Intestine, faeces, meat
	Non-O157 EHEC	O26, O111, O103, O113, O145	Worldwide	Cattle, shoats, pigs, chickens	milk, cheese, Intestine, faeces, meat
Potentially zoonotic	None	O17, O56, O87, O108, O109, O130, O136, O149	Worldwide	Cattle, shoats, pigs,	Intestine, faeces, meat
Animal pathogenic	EDEC	O138, O139, O141	Worldwide	Pigs	Intestine

(Sources: Fairbother and Nadeau, 2006)

2.4. Sources of Escherichia Coli Infections

Escherichia coli can enter a dairy farm environment through new herd members; environmental media such as air, water, and soil; wildlife; or organic materials, such as cattle feed and bedding (Fairbother and Nadeau, 2006). Once an *E. coli* strain has entered the herd, it can persist in the animals' intestines and be excreted in the environment (Evans *et al*., 2000). However, the dynamics and routes of introduction, colonization, and persistence in both animals and the farm environment are not well characterized (Renwick *et al*., 1993). The dynamics and routes of spread of genetic elements associated with STEC and EHEC virulence in dairy herds and farm environments are also poorly understood (Heuvelink and Bleumink, 1998).

Escherichia coli O157:H7 bacteria and other pathogenic E. coli is believed to mostly live in the intestines of cattle (Elder, *et al*., 2000) but has also been found in the intestines of chickens, deer, sheep, and pigs. As Keen *et al*., (2003) reported on the prevalence of E. coli O157:H7 in livestock at 29 county and three large state agricultural fairs in the United States found that E. coli O157:H7 could be isolated from 13.8% of beef cattle, 5.9% of dairy cattle, 3.6% of pigs, 5.2% of sheep, and 2.8% of goats. Over seven percent of pest fly pools also tested positive for *E. coli* O157:H7 (Keen *et al*., 2003). Shiga toxin-producing *E. coli* does not make the animals that carry it ill. The animals are merely the reservoir for the bacteria (Fairbother and Nadeau, 2006).

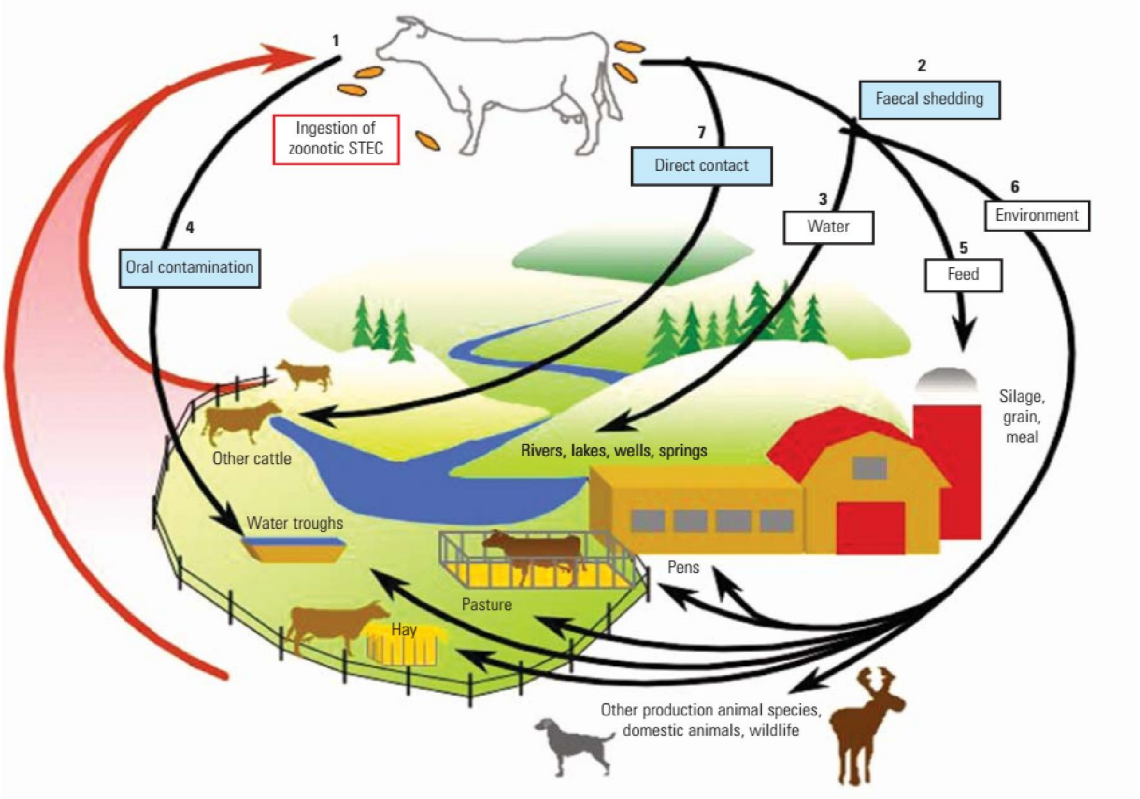

Figure 2: sources of zoonotic STEC infection in farm animals.
(Sources: Fairbother and Nadeau, 2006)

2.4.1. Animal reservoirs and exposure assessment

Shiga toxin E.coli can be found in the gut of numerous animal species, but ruminants have been identified as a major reservoir of STEC that are highly virulent to humans, in particular EHEC O157. Knowledge about the routes of transmission and the origin of human infections has regularly improved during the past twenty years, as numerous epidemic events have been investigated. So, it seems evident that STEC may be transmitted from animal reservoirs to humans not only via the ingestion of contaminated foods or drinking water, but also by contact with STEC-positive animals or with their environment (Pennington, 2010). Cattle are considered to be the most important source of human infections with EHEC O157, being asymptomatic excretors of the organism, which is a transient member of their normal gut microflora. The presence of EHEC O157 in cattle excreta appears to be influenced by the age of the animals (Fairbrother and Nadeau, 2006).

Humans are infected with zoonotic STEC mostly through the consumption of foods contaminated with faeces containing the bacteria (Rangel *et al*., 2005). Food has remained the predominant transmission route: the most important food sources being undercooked hamburgers and ground beef products. Raw milk and milk products, such as cheese curds, butter, and ice cream bars, have also been a source of infection. Since 1991, produce has been an increasingly important cause of outbreaks: high risk products include lettuce, unpasteurised apple cider and juice, salad, coleslaw, melons, and sprouts. Outbreaks of O157 STEC most commonly occurred in restaurants, often due to cross-contamination during food preparation. Person-to- person transmission via the faecal-oral route has been an important mode of transmission, particularly since the early 1990s, and occurs mostly in child day care centres, individual homes, communities, and schools (Pennington, 2010). Waterborne outbreaks of O157 STEC associated with recreational waters, such as lakes, swimming pools, and contaminated drinking water, have been increasingly reported since the early 1990s (Fairbrother and Nadeau, 2006).

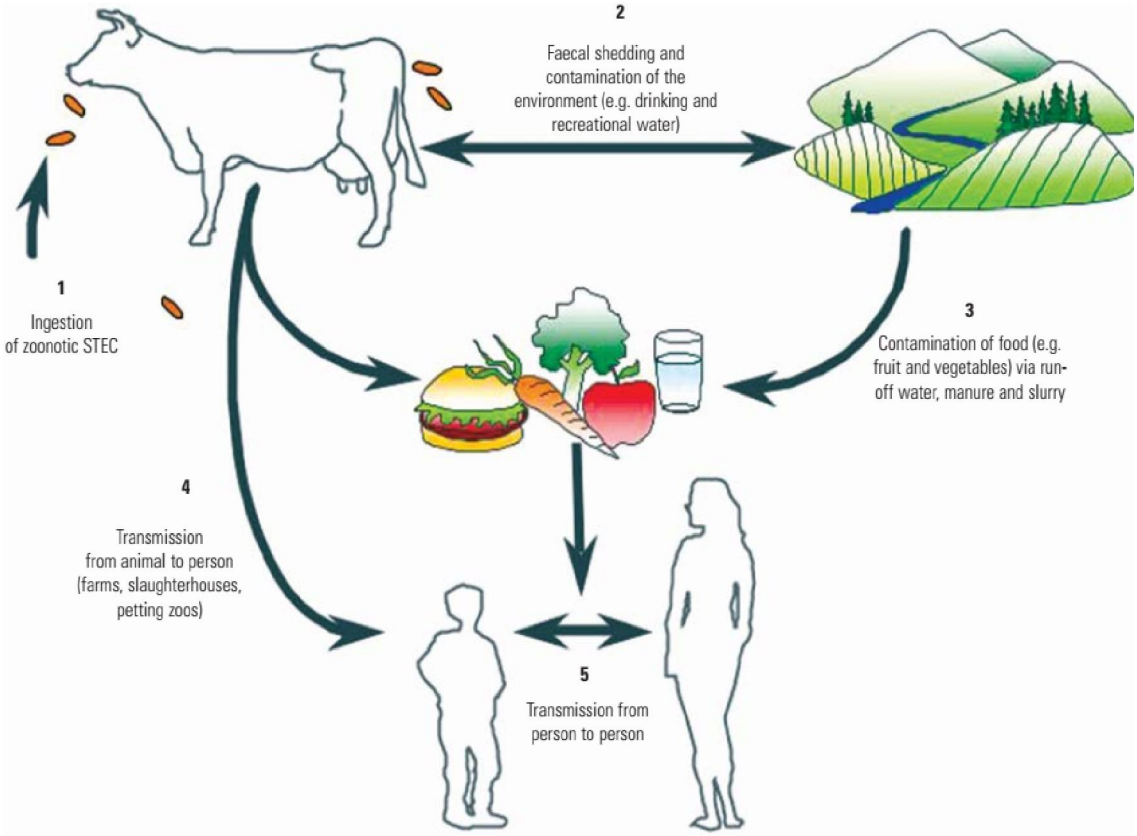

Figure 3: How humans are exposed to zoonotic Shiga toxin producing Escherichia coli.
Source: (Fairbrother and Nadaeu, 2006)

2.5. Zoonotic Potential and nature

Shiga toxin Escherichia coli are a serious human pathogen but except in greyhounds and in some very young animals, it has not yet been recognized as a significant animal pathogen (Fairbrother and Nadaeu, 2006). The evidence of zoonotic transmission of STEC was associated with consumption of unpasteurised milk and dairy products (Farrokh *et al.,* 2013). However it has enormous veterinary public health significance as many reports indicate that livestock are the reservoir of STEC (Synge, 2000). Contact with animal faeces (Evans *et al.,* 2000) or direct contact with animals such as calves (Renwick *et al*., 1993; Synge *et al*., 1993) or lambing ewes (Allison *et al*., 1997) is well-established risk factor for STEC acquisition. As Martin and Beutin (2011), stated that it is difficult to explain why only few humans are affected when the prevalence of STEC is very high among cattle. EHEC and EAHEC are important causes of illness in people. Why some organisms regularly cause illness in people, and others are found rarely or not at all, is still uncertain. Humans are the only known reservoir hosts for EAEC and related species such as EAHEC O104:H4 (https://www.efsa.europa.eu/en/efsajournal/pub). The existence of two distinct lineages of STEC as detected by an octamer-based genome scanning system that identifies a population of STEC found in cattle but not found in man (Kim *et al*., 1999; Fairbrother and Nadaeu, 2006).

2.5.1. Risk factors for infection of animals and humans

Risk factors that have been associated with the infection of animals with O157 STEC include age, weaning, movement of animals, season, feed composition, and the ability of the bacteria to persist in the environment. Faecal shedding was higher in dairy calves at weaning than before weaning (Jo MY *et al*., 2004, Rangel *et al*., 2005). The ability of zoonotic STEC to survive and persist in faeces, manure, and soil in the environment can be considered as a risk factor for the infection of animals and humans. It has been shown that O157 STEC can survive for several months in water or sediment from drinking troughs (Kudva and Hovde, 1998). These bacteria can also survive for long periods in cattle faeces, particularly when the moisture content remains high (Wang and Doyle, 1996), and in cattle or sheep

manure piles and manure slurry (Kudva and Hovde, 1998; Jo MY *et al.,* 2004).

2.5.2. Host factors that influence disease

People of all ages are susceptible to infection with STEC. However, the young and the elderly are more susceptible and are more likely to develop more serious symptoms (FDA, 2012). The dose response relationship for STEC is complicated by the number of serotypes and the association of STEC with a variety of foods. The infective dose of E. coli O157:H7 is estimated to be very low, in the range of 10–100 cells. The infective dose of other STEC serotypes is suspected to be slightly higher (Fairbrother and Nadaeu, 2006; FDA, 2012). Dose response models have been developed for *E. coli* O157:H7. Teunis *et al*. (2004) used data from an *E. coli* O157:H7 outbreak at a school in Japan to estimate the dose required to cause disease. In children the estimated ingested dose was 31 organisms, with 25% of exposed children becoming ill. In adults the estimated ingested dose was 35 organisms, with 16% of exposed adults becoming ill. As Haas *et al.* (2000) reported that data used from a prior animal by Pai *et al*. (1986) and validated their model by comparison with two human outbreaks, one foodborne and the other waterborne that occurred in the US. This model approach estimated that the dose required for 50% of the exposed population to become ill was 5.9 $x10^{-5}$ organisms. The corresponding probability of illness for the ingestion of 100 organisms was 2.6 x10-^{4} (Pennington, 2010).

Human feeding trial data has been used to generate a dose response model for *E. coli* serotypes other than E. coli O157:H7 (*E. coli* O111 and O55) (Haas *et al*., 2000). The model estimated the dose required for 50% of the exposed population to become ill was 2.55 $x10^{6}$ and the probability of illness for ingestion of 100 organisms was 3.5 x 10^{-4}. There is no clear relationship between feed composition and STEC faecal shedding in cattle. Some authors formulated the hypothesis that a grain-rich diet may induce mechanisms of STEC acid resistance in the rumen that favour STEC survival and faecal shedding (Meyer *et al.,* 2001; Pennington, 2010).

2.4.3. Virulence and infectivity

Shiga toxin Escherichia strains produce two types of Stx (Stx1 and Stx2). Stx1 is virtually identical to the toxin produced by Shigella dysenteriare serotype1. The presence of Stx2 is significantly associated with human disease. These two Stx immunologically non-cross reactive groups called Stx1 and Stx2 (Fairbrother and Nadaeu, 2006; Spears *et al*., 2006). Shiga toxins are toxic to Vero cells (African green monkey kidney cells) and so are also known as verotoxins. The term STEC is used interchangeably with VTEC. In the laboratory, Vero cells can be used to detect shiga toxin activity, as shiga toxin causes Vero cell death (Desmarchelier and Fegan, 2003; Meng and Schroeder, 2007). Due to the acid resistance of STEC, when ingested it is able to survive in the stomach environment and attach to the cells of the intestine. Some STEC strains form a characteristic attaching and effacing lesion on the intestinal cells. The presence of these lesions is a risk factor for the development of HUS (Gyles, 2007). Stx produced by STEC is able to bind to specific receptors on susceptible host cells, resulting in the death of these cells. Vascular endothelial cells are a primary target for Stx. Hence production of sufficient Stx results in damage to the blood vessels in the colon and subsequent bloody diarrhoea. Sufficient Stx is taken up by the blood and circulated through the body, this can lead to impaired kidney and neurological function and the development of HUS (Desmarchelier and Fegan 2003; Gyles 2007).

2.6. Contamination of Food Pathways

The epidemiology of foodborne pathogenic *E. coli* varies throughout the world, which are excreted in the feaces of either ill or healthy hosts. In communities with poor sanitation and hygiene, ETEC, EIEC and EPEC are prevalent. They are acquired by consumption of contaminated food and water and by cross-contamination through direct human contact. Foodborne pathogenic *E. coli* have emerged paradoxically in communities with better developed sanitation and hygiene. However, the pathotype differ STEC, EHEC and EAggEC and the transmission pathways often include raw or inadequately processed animal or horticulture products, contact with animal manure, contaminated water and cross-contamination with raw food (Fairbrother and Nadaeu, 2006). Ruminants and wildlife appear to be major reservoirs of STEC and EHEC, while the human host may be more important for other pathotypes. Because of the wide dissemination of human and animal faecal material into the environment, the bacteria have the potential to be present in areas used for food production (Fairbrother and Nadaeu, 2006; FAO, 2009). A wide range of foods may be a vehicle for pathogenic *E. coli* in association with their respective ecologies. Food may be contaminated and/or cross-contaminated during growth and harvest (horticulture products), collection (milk) or slaughter (meat).

The recent and apparently sudden emergence of STEC and its association with an increasingly wide range of foods have resulted in this group of organisms being a major focus for the food industry. Ground beef, so far, has been found to be major vehicle for STEC transmission (Acheson, 2000). Although, there is much focus on O157:H7, the pathogenic roles of other serovars are gradually being recognized preparation (FAO, 2004; Franz and Bruggen, 2008).

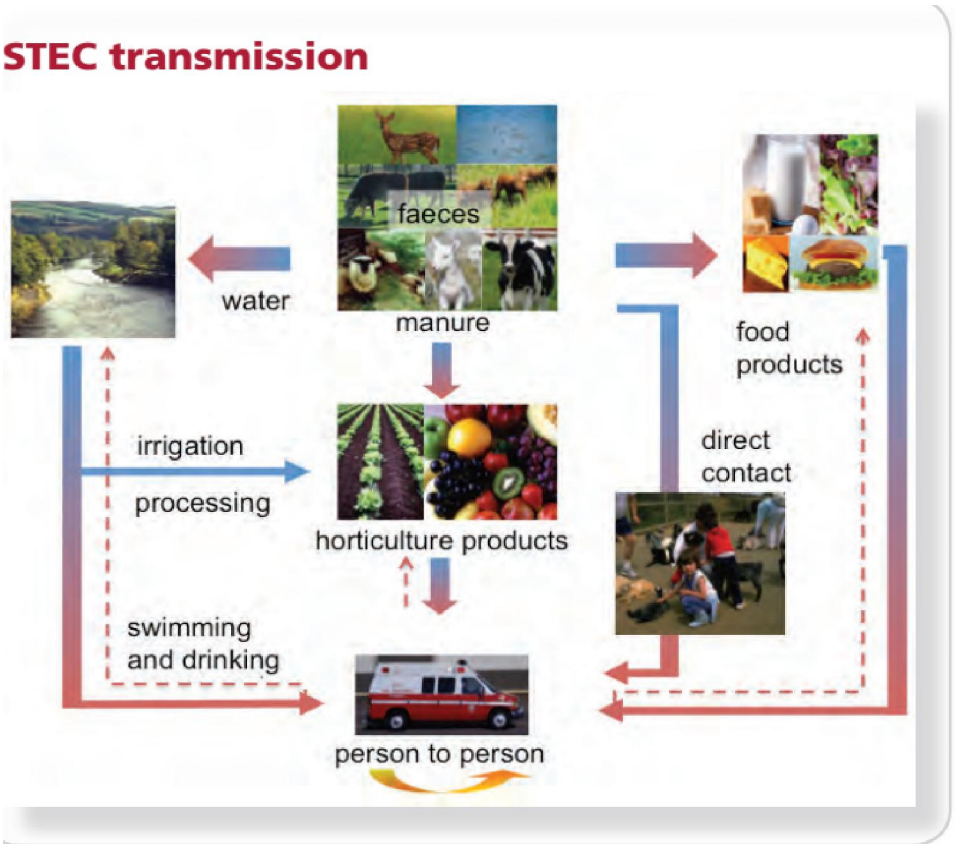

Figure4. The many ways food can get tainted from farm to fork
Source: Jeffrey Lejeune

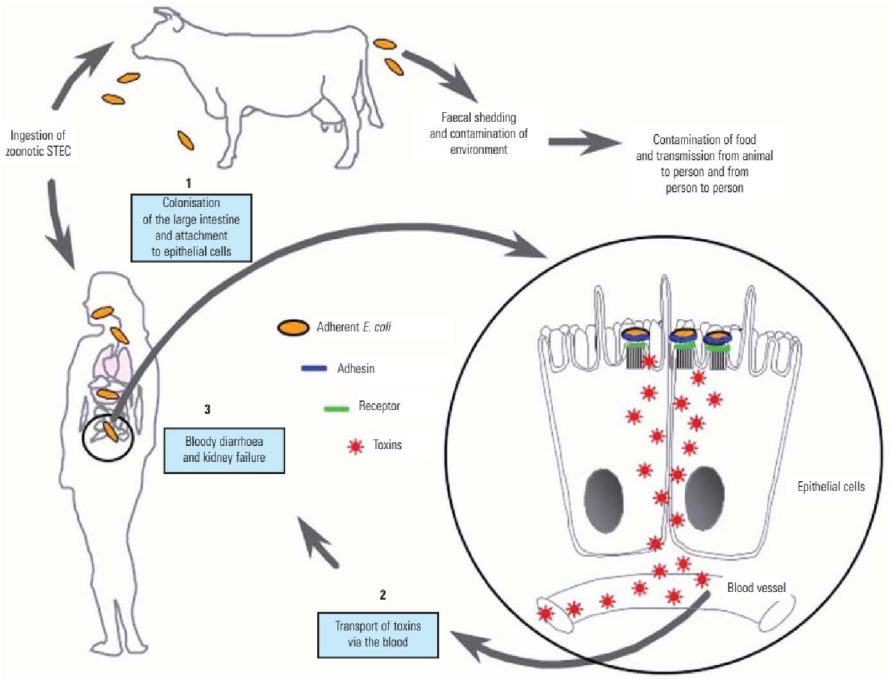

Figure 5: How zoonotic STEC cause bloody diarrhea and HUS in human

2.7. Pathogenesis

Production of a potent Stx is essential for many of the pathological features as well as the life threatening sequelae of STEC infection (Karmal *et al*., 2010). However, pathogenesis is a multistep process, involving a complex interaction between a range of bacterial and host factors. Orally ingested STEC (often in very low initial doses) must initially survive the harsh environment of the stomach and then compete with other gut microorganisms to establish intestinal colonization (Meng and Schroeder, 2007). STEC organisms remain in the gut, and so STX produced in the lumen must be first absorbed by the intestinal epithelium and then translocated to the bloodstream. This permits delivery to the specific toxin receptors on target cell surfaces inducing both local and systemic effects (Fairbrother and Nadaeu, 2006).

2.8. Clinical Signs and Symptoms of Disease

Infection with STEC can result in no clinical symptoms (asymptomatic infection) or can cause diarrhoea (may progress to bloody diarrhoea), abdominal cramps, vomiting and fever. The onset of illness is 3–8 days (median of 3–4 days). Most patients recover within 10 days of the initial onset of symptoms (Meng and Schroeder, 2007; WHO, 2011). In some cases, patients develop HUS. HUS is characterized by hemolytic anemia, thrombocytopenia (decrease in blood platelets) and kidney failure. HUS can also have neurological effects and cause seizures, stroke and coma (WHO 2011). Approximately 6.3% of STEC infected individuals develop HUS, with a fatality rate of 4.6%. Children are more susceptible, with 15.3% of children under five years of age developing HUS following STEC infection (Gould *et al*., 2009). STEC are shed in the faeces of infected individuals for several weeks. In children the median shedding time is 13 days (range of 2–62 days) for individuals with diarrhoea. In people who develop HUS, the median shedding time is 21 days (range 5–124 days) (Meng and Schroeder 2007; Pennington, 2010).

2.9. Mechanisms of Resistance

The use of veterinary drugs in food-producing animals has the potential to generate residues in animal products milk, meat and others that poses a health hazard to the consumer (Beyene, 2016). Antimicrobial resistance is a major and increasing global healthcare problem (WHO, 2012). Increased consumption of antimicrobial agents and their inappropriate use are among factors which further accelerated this phenomenon (Vander and Pitou, 2012). Resistant bacteria from animals can infect humans by direct contact as well as via food products of animal origin (Szmolka and Nagy, 2013). *Escherichia coli* are intrinsically resistant to therapeutic levels of penicillin G, the first β-lactam introduced into clinical practice, because of its outer membrane barrier. Escherichia coli is also resistant to several different classes of antibiotics with distinct mechanisms of action like β-lactams, quinolones and aminoglycosides, because the invasive E. coli was mainly resistant to their action as reported (CDC, 2012).

2.10. Diagnostic method in Animals and Human

Carrier animals are usually detected by finding EHEC in fecal samples, which are either freshly voided or taken directly from the animal. Rectoanal mucosal swabs are useful for some purposes, but seem to detect fewer infected animals. Repeated sampling, as well as sampling more animals, increases the chance of detection (Gould *et al*., 2009). EHEC can also be found in other locations, such as hides or dust, and animals are not sampled routinely for EAHEC. EHEC can be difficult to identify in animals. They are a minor population in the fecal flora, and they closely resemble commensal *E. coli* except in verotoxin production. There is no single technique that can be used to isolate all EHEC and EAHEC (Mora *et al*., 2009).

Because humans do not normally carry EHEC, clinical cases can be diagnosed by finding these organisms in fecal samples. Samples should be collected as soon as possible after the onset of diarrhea, as these bacteria may be cleared after a week. There is relatively little information yet about EAHEC; however, some people seem to shed EAHEC O104:H4 subclinically for a prolonged period after recovery (Pollock *et al*., 2009). The techniques to identify EHEC and EAHEC are similar to those used in animals. These tests include dipstick and membrane technologies, agglutination tests, microplate assays, colony immunoblotting, PCR, immunofluorescence and ELISAs. Fecal samples can be tested directly with some tests, but sensitivity is improved by testing cultures (CDC, 2016).

2.11. Treatment of Infection

In most infected individuals, symptoms of *E. coli* infection last about a week and resolve without any long-term problems. As Jelacic and Tarr (2000) reported antibiotics do not improve the illness, even some medical researchers believe that these medications can increase the risk of developing HUS. So, apart from good supportive care such as close attention to hydration and nutrition, there is no specific therapy to halt *E. coli* symptoms. The recent finding that *E. coli* O157:H7 initially greatly speeds up blood coagulation may lead to future medical therapies that could forestall the most serious consequences (Chandler, *et al*., 2002). Most individuals who do not develop HUS recover within two weeks. Treatment for those who develop HUS ranges from mild to very intensive. Children are generally in the hospital for

about two weeks (range 3 days to 3 months), and adults longer, as their courses tends to be more severe. Since there is no way to end D+HUS, supportive therapy, including meticulous attention to fluid and electrolyte balance, is the cornerstone of survival (Fairbother and Nadeau, 2006; www.about-hus.com).

2.12. Control and Prevention Strategies

As for other zoonotic agents, having animals and raw products that are free from STEC is not possible in practice (Fairbother and Nadaeu, 2006). As far as the transmission through the direct contact with animals is concerned, both farmers and people visiting farms should apply hygiene practices (Alfredo *et al*., 2004). However, their occurrence can be minimized by applying high standards of hygiene in all the steps of the food production chain. At the farm level, classical eradication strategies based on the elimination of positive animals are not feasible, due to the high prevalence of colonisation, its transient nature and the technical difficulties in detecting low levels of the organism in animal faeces (Thran *et al*., 2001). Many approaches have been attempted to reduce the intestinal colonisation in cattle. These include interventions on the diet of the animals, the administration of probiotics as competitive microflora (Brashears *et al*., 2003) and the use of bacteriophages active on EHEC O157 (Alfredo *et al*., 2004).

Prevention, Because EHEC are not usually significant pathogens in animals, preventive measures are mainly intended to reduce carriage for the benefit of humans. How best to accomplish this is still unclear. Identifying and targeting super-shedders has been proposed as a particularly effective means of control; however, the effects of such measures and methods to identify super shedding animals are still debated. Vaccines against EHEC O157:H7 may reduce shedding, and have received full or conditional approval in some countries including the U.S. and Canada, but are not in wide use. Other proposed interventions include the application of disinfectants (chlorhexidine), various antimicrobials or bacteriophages to the terminal rectum; the use of probiotics that would preferentially colonize the gastrointestinal tract; dietary manipulations; reductions in animal density in feedlots to decrease transmission rates; and hygiene/ management measures such as the provision of dry bedding, frequent cleaning of water troughs and the grouping of animals in the same cohorts through each stage of growth. These interventions are generally still in the research stage, although some appear promising. In addition, animals should not be allowed to graze pastures for a period after effluent that may contain EHEC has been applied (Thran *et al.,* 2001; Fairbother and Nadaeu, 2006).

2.13. Alternative Therapies

The worldwide emergence of multidrug-resistant bacteria has dramatically limited the number of antibiotics that retain activity against these pathogens. This problem has been further amplified by the dearth of novel classes of antibiotics. Therefore, development of novel therapeutic strategies for infectious diseases is high demand. In response, several new therapies have been developed, such as phage therapy, antimicrobial peptide therapy and combinations of two or more antibiotics (Fjell *et al*, 2012, Haq *et al,* 2012). The potential use of bacteriophages as therapeutic agents was recognized from the 1900s (Haq *et al*, 2012). However, this therapeutic approach was eclipsed by the discovery and use of antibiotics. Nevertheless, phage therapy was used for the treatment of human bacterial infections, mainly in Eastern Europe (Abedon *et al,* 2011). Recently, the rise of multidrug-resistant bacteria and the consequent decrease in the number of effective antibiotics has forced scientists to search for alternative therapies (Haq et al, 2012). Phages have a number of advantages that make them attractive for therapeutic use against bacteria. First, they are highly specific and can be very effective in lysing bacteria. Second, phages are safe as underscored by several clinical studies, and third, they can be readily modified to fight the emergence of new multiresistant bacterial strains (Sulakvelidze *et al*. 2001. Many studies characterizing lytic phages specific for different *E. coli* strains have been published demonstrating their potential therapeutic value (Maura *et al*, 2012, Sillankorva *et al.,* 2012).

2.14. Escherichia Coli as Biological Weapon

E. coli is present in the CDC list of biological agents potentially threat to public health and safety. Several microorganisms or their products can be used as biological weapon for warfare and bioterrorism. In Category A agents which can be easily disseminated or spread from person to person, resulting in high mortality rate and impact on public health are listed (CDC, 2013). Category B lists pathogens moderately easy to disseminate, resulting in moderate morbidity rates and low mortality rates. Category C lists emerging pathogens with potentially high morbidity and mortality and which can be engineered for mass dissemination. E. coli O157:H7 strain is present in Category B as “food safety threat”. *E. coli* O157:H7 strain is present in Category B as “food safety threat”. Even though less dangerous than Category “A” agents, Category “B” agents are easier to produce and handle, and the use of such agents against civilian populations by terrorists might well cause considerable panic (Anderson and Bokor, 2012). It is considered as the major indicator of fecal pollution in food production. Its presence in processed foods results from recontamination, because this bacterium usually does not survive food preservation processes. The main

reasons for the presence of E. coli in food products are nonobservance of relevant technological regimes, incompliance with recommended process standards, and the lack of personal hygiene (Law, 2000).

2.15. Public Health Impact of *Escherichia coli*

The majority of E. coli rods do not constitute a serious health hazard, but some serotypes can cause food poisoning and alimentary intoxications. The most dangerous among them are EHEC strains, especially serotype O157:H7. E. coli O157:H7 has become a pathogen of major concern in both food and dairy industries, and to the public, because of its ability to cause severe illness, in particular, haemorrhagic colitis, HUS and TTP (Reuben *et al.,* 2013). The sources of infections with EHEC strains are mostly meat products, especially underdone steaks and hamburgers (Chinen *et al.,* 2001), but also other foodstuffs as unpasteurized milk and dairy products manufactured from raw milk, have been implicated in many outbreaks, (Maher *et al*., 2001).

3. Conclusions And Reccommendations

Many milk borne epidemics of human diseases are spread through milk contamination, which harbor a variety of microorganisms and can be important sources of food borne pathogens. The presence of food borne pathogens in milk may be due to direct contact with contaminated sources in the dairy farm environment and to excretion from the udder of an infected animal. Shiga toxin producing E. coli are foodborne pathogens that may cause serious illness in humans. Among the food related zoonoses STEC are bacteria that form part of the normal gut flora of humans and other warm-blooded animals. Although most E. coli are considered harmless, certain strains can cause severe illness in humans. Infection with STEC is the main cause of HUS, a condition which can be fatal in humans. Infection of humans is mostly caused by the consumption of infected foodstu□s derived from bovine animals, such as milk and milk products. Zoonotic pathogens, such as E. coli O157, which have animal reservoirs, also have direct impact on public health. Great strides have been made in recent years in identification and characterisation of O157:H7 STEC, which has led to a more accurate assessment of the role of this serotype in human disease outbreaks and the transmission of infection from animal reservoirs. A major challenge will now be to better understand how these bacteria colonise the gut of the animal hosts Such an understanding will permit the development of effective strategies to eliminate and reduce the numbers of the bacteria in the animal reservoir, new food vehicles are questions and problems for food industries and public health agencies.

✓ Emphasises the need for effective and continuous efforts on the safety and health issues related to raw milk hazards.

✓ Educational efforts to improve dairy farmer's awareness of milk borne zoonoses, risk factors associated with milk borne pathogens, efficient cleaning of all utensils and equipment and the consumers should take in consideration the cleanliness of sales persons.

✓ The microbiological health hazard arising from the consumption of contaminated high risk food like milk has grown in recent years and has result in national and international intensification of food hygiene programs.

✓ It is of outmost importance to examine the stool specimens of apparently healthy dairy handlers to clarify their role in shedding bacterial pathogenic agents and protect public health, more stringent regulations and strategies are in demand.

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