

Epidemiology and control of *E. coli* O157 infection

Abstract

E. coli is a commensal of the digestive tract of all animals. However, there are some pathogenic strains of *E. coli* that causes illness in humans. These pathogenic *E. coli* are classified as enteropathogenic (EPEC), enterotoxigenic (ETEC), enterohaemorrhagic (EHEC) or verocytotoxigenic or verotoxigenic (VTEC), enteroinvasive (EIEC), enteroaggregative (EAEC) and diffusely adherent (DAEC) *E. coli*. *E. coli* O157: H7 is the most notable strain of EHEC that produces Shiga-like toxins causing haemorrhagic colitis (HC) in humans, which may progress to haemolytic uraemic syndrome (HUS) or thrombocytopenic purpura (TTP). These are potentially life-threatening illness, particularly in children and elderly people. Renal failure is the most important sequela of these complications.

Cattle are the natural reservoir of *E. coli* O157: H7 with a prevalence rate of more than 35%. Various food items contaminated with cattle faeces are implicated as the source of human infections, beef and beef derived products being the most important. Also, the farm environment is an ideal reservoir harbouring this pathogen.

Since 1982, infection caused by this pathogen has emerged as a major public health threat as large outbreaks have occurred in many countries, including the United States, United Kingdom, Japan and some other countries. In spite of the stringent control measures, there is no significant decrease in the incidence of outbreaks. This may be due to failure of coordination in implementation of control measures at all levels of the production chain.

At the present moment, control measures at the producer's level should be aimed either at reduction in herd prevalence of infection, reduction of concentration of *E. coli* O157 in faecal shedding, reduction in amount of soiling transferred from the hide to the carcass, or a reduction in opportunity for cross-contamination in the processing plants. The recommended control measures for prevention of *E. coli* O157 infection at consumer's level include the need to avoid eating raw or undercooked ground meats (milk) and prevent cross-contamination in the kitchen and to wash hands, utensils, and preparation surfaces that have come in contact with raw meat.

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1 Introduction

Escherichia coli is a commensal of the digestive tract of all animals, including humans. The organism serves an important function in the body by synthesizing valuable vitamins, and by competitively inhibiting the growth of pathogenic bacteria that may be ingested with food and water. *E. coli* is being used, since many years, as an indicator of faecal contamination, because of being one of the dominant species in human faeces. *E. coli* is referred to as opportunistic bacteria as it usually cause infections in debilitated and immunocompromised individuals. Nevertheless, another group of these bacteria, referred to as pathogenic *E. coli* (EC), are capable of causing diarrhoeal illness even in healthy individuals because of their distinctive virulence factors. These pathogenic *E. coli* are classified into six major categories: enteropathogenic (EPEC), enterotoxigenic (ETEC), enterohaemorrhagic (EHEC)/verocytotoxigenic or verotoxigenic (VTEC), enteroinvasive (EIEC), enteroaggregative (EAEC) and diffusely adherent (DAEC) *E. coli*.

It has been documented that there are more than 200 serogroups of EHEC that produce Shiga-toxins (Stx), and a number of these have been associated with sporadic human infections and outbreaks (Coia, 1998; Feng, 2001). However, *E. coli* O157:H7 remains the most important of the EHEC group of organisms (Callaway et al. 2003) which causes hemorrhagic colitis (HC). In about 3-7% of the cases (Feng, 2001), HC may progress to serious complications, such as haemolytic uraemic syndrome (HUS) especially in children, and thrombocytopenic purpura (TTP) and death (Dundas et al., 1999).

E. coli O157:H7 is a foodborne bacterial pathogen of great public health concern due to the increasing number of outbreaks and high mortality rate associated with this organism (Elliott and Nichols, 2001). Infection with this organism was first described in 1982 (WHO, 2002). Various food types have been implicated in *E. coli* O157:H7 outbreaks, including apple juice (Besser et al., 1993), fruits and vegetables (Chapman et al., 1997b), alfalfa sprouts (Gordon and Jenkins, 1998) and milk (Coia, 1998), but beef and beef products remain the most common vehicles for infection by this pathogen (CDC, 2002). The infective dose of *E. coli* O157:H7 may be as low as 10-100 cells (Feng, 2001) and infected individuals may shed the organism for up to 87 days, resulting in a risk of person to person transfer (Chapman et al., 1997b). For these reasons many countries have

imposed strict regulations and controls on the presence of this pathogen in foods and food processing environments.

Although the number of infections are very small as compared to other foodborne infections like *Salmonella* and *Campylobacter* (Coia, 1998, Mead et al., 1999), it is well-known that *E. coli* O157:H7 infection may be life-threatening. VTEC strains of serogroup O157 have caused very large outbreaks of food poisoning, often with significant morbidity and mortality. These have occurred in several different parts of the world, particularly in the developed countries like USA, UK and Japan. This essay provides a timely review of VTEC, particularly O157: H7, in relation to foodborne disease and details the hazards specifically in relation to meat and meat products, and how these may be controlled on farm, and at processing and protection of the consumers against infection.

2 The organism/toxin

2.1.1 Microbiological characteristics

E. coli O157 is facultatively anaerobic, non-spore-forming, Gram-negative rod; more than 80% carbon dioxide is inhibitory. Motility is seen in most of the strains. *E. coli* have been divided into 170 serogroups based on the somatic (O) antigens, in addition to 50 serogroups based on flagellar (H) and about 100 serogroups based on capsular (K) antigens (Bells and Kyriakides, 1998). The predominant serotype O157: H7 is designated based on the flagellar antigens.

Although the morphological features and biochemical reactions of the serotype O157:H7 are typical of *E. coli*, this serotype can not ferment sorbitol rapidly (<48 h) (Neill et al., 2001; Vernozy-Rozand et al., 2002). This had led to the development of sorbitol MacConkey agar (SMAC) as a selective screening medium. The most commonly followed method is selective culture of faeces in broth media followed by immunomagnetic separation (IMS) and subculture on to selective and differential media, such as cefixime tellurite sorbitol MacConkey agar (CT-SMAC) (Chapman, 1999). The IMS enrichment technique involves the use of immunomagnetic beads coated with an antibody against the O157 antigen to extract *E. coli* from faeces. These techniques are

very sensitive and can detect as few as 1 CFU *E. coli* O157: H7 per gram (Gannon, 1999). The optimum temperature of growth for *E. coli* O157:H7 have been found to 37°C (Bell and Kyriakides, 1998).

Toxins: *E. coli* O157 produces two different toxins: VT1 and VT2, together with a small number of VT variants. Approximately 60% sequence homology is shared by VT1 and VT2. VT1 is related structurally and biochemically to the Shiga-toxin of *Shigella dysenteriae*, and the only difference is in single amino acid (Coia, 1998). One or more Shiga-toxins are produced by the serotype *E. coli* O157: H7.

2.1.2 Characteristics significant to its control

E. coli O157:H7 have been found to grow in a wide range of temperatures: 8°C being the minimum, 44-45 °C the maximum, and 37°C being the optimum temperature (Bell and Kyriakides, 1998; Coia, 1998).

Once in food or food ingredients, *E. coli* O157: H7 develops a remarkable ability to survive some of the conditions and processing procedures followed in the food industry. Although it does not actively multiply, the organism can survive at refrigerator and freezer temperatures (Coia, 1998; Bell and Kyriakides, 1998). In ground beef, the organism has the ability to survive up to 9 months in frozen storage at -20°C (Feng, 2001).

High acid tolerance is exhibited by some strains of *E. coli* O157:H7 (Bolton et al., 1999; Zhao et al., 1998). They do not grow well at low pH but can survive low pH and resume growth if the pH is again favourable (Russell et al., 2000). The organism has been found to survive for up to 56 days at pH \geq 4.6 (Feng, 2001). They also survive the fermentation and drying process used in preparation of foods (CDC, 1995).

However, *E. coli* O157 are very much heat sensitive, and are readily killed by normal cooking and pasteurization procedures (Feng, 2001). Thermal inactivation studies revealed that, in ground beef, the time required to reduce 10% of initial population of *E. coli* O157: H7 (*D* value) at 64. 5°C was only 9.6 s (Feng, 2001). Thus, cooking and pasteurization of foods seems to be the most reliable and effective measures to control these pathogens. The toxin produced is, however, stable which can survive the cooking

conditions set for ground beef, but the role of these toxins in producing illness is not clearly established (Feng, 2001).

Modified atmosphere packaging with combination of mixtures of CO₂, O₂ and N₂ have no inhibitory effect on the organism (Feng, 2001).

E. coli O157 is relatively sensitive to irradiation and can be eliminated from foods, including beef, by low-dose treatments (Farkas, 1998).

3 Epidemiological features

3.1 Reservoirs/Sources

3.1.1 Human

Infection with *E. coli* O157 may produce an asymptomatic carrier state in humans and may contribute to subsequent secondary person-to-person transmission (Chapman et al., 1997b; Coia, 1998; Feng, 2001). Reports on the length of the period of excretion of *E. coli* O157 following infection differ widely. Prolonged excretion of the organism, for up to 89 days after infection, has been reported by Chapman et al. (1997b). (Detail discussion in section 3.2).

3.1.2 Animal

E. coli O157:H7 exists in a wide range of farm animals including cattle, sheep, pigs and deer (Table 1). Poultry meat is also implicated as a possible risk factor for infection by *E. coli* (Chapman et al., 1997a). However, cattle with the prevalence rate of up to 36.8% (Chapman et al., 1997a) are generally regarded as the major reservoir for human infection (Coia, 1998). The organism is considered to be non-pathogenic in cattle (Brown et al., 1997). In one study in calves, the primary sites of localization of the organism were found to be the rumen and omasum, and parts of intestine distal to the ileum (Brown et al., 1997). In cattle, the pathogen has been detected in faeces, hide, and derived carcasses (Chapman et al., 1997a; Cagney et al., 2004). The prevalence rate of *E. coli* O157 in dairy cattle is slightly higher than in beef cattle (16.1% versus 13.4%) (Chapman et al., 1997a). Although VTEC has been detected in pigs (Eriksson et al., 2003) and pork (Fantelli and Stephan, 2001), their role in epidemiology of humans infections is not

clearly established (Bouvet et al., 2001). It is suggested that pigs should also be considered as a potential reservoirs for human infections since similar PFGE pattern was seen in *E. coli* O157: H7 isolated from ruminants and pigs that were reared in a Swedish farm (Eriksson et al., 2003). In this study, the organisms were believed to be transmitted to the pigs from the ruminants via the workers' clothing and boots.

Table 1. Isolation rate of *E. coli* O157 from faeces of different animal species

Animal	Country	Year	Isolation rate	Reference
Beef cattle	Canada	1999	12.4%	Van Donkergoed et al. cited by Gannon, 1999
Cattle (beef & dairy)	United Kingdom	1995-1996	15.7% of 4800	Chapman et al., 1997a
Cattle	Netherlands	-----	10-11%	Reinders et al., 2001
Sheep	United Kingdom	1995-1996	2.2% of 1000	Chapman et al., 1997a
Pig	Sweden	1997-1998	.08% of 2446	Eriksson et al., 2003
Pig	United Kingdom	1995-1996	0.4 % of 1000	Chapman et al., 1997a
Dairy heifers	USA	1999	9.5% of 91	Thran et al., 2001
Calves (age 68-227 days)	Italy	1996-1997	10.7% of 1293	Conedera et al., 2001
Zebu cattle	Uganda	1998	28.3% of 159	Kaddu-Mulindwa et al., 2001

Seasonal trend is usually observed in the isolation rate of this organism from the cattle faeces. In one Canadian study, lowest isolation rate of 0.75% of all cattle at slaughter was found in the winter (December-February) and highest of 19.7% of all cattle in the summer (June-August) (Gannon, 1999). Similarly, in a study in the United Kingdom, Chapman et al. (1997a) found this seasonal variation (4.8 to 36.8%) in isolation rate, with highest rate in the spring (May) and late summer (figure 1). Similar was the finding of an Italian study (Conedera et al., 2001). In contrast to these findings, study in the USA (Thran et al., 2001), in a population of dairy heifers, found prevalence of 9.5% only during the winter months.

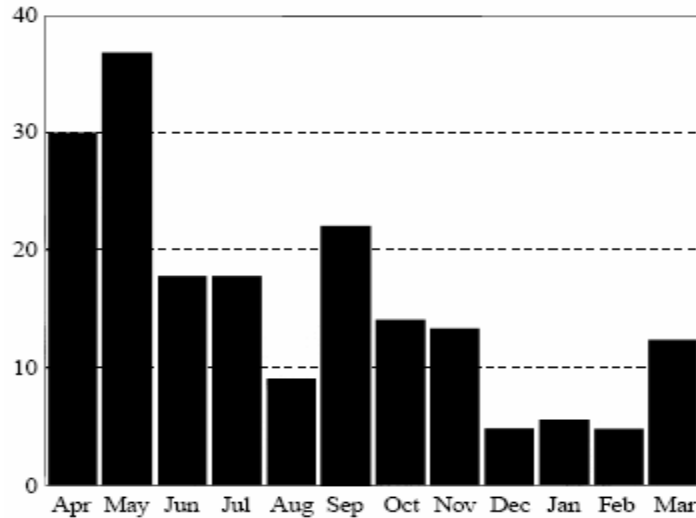


Figure 1. Seasonal distribution (%) of *E. coli* O157 isolates from cattle in the United Kingdom, 1995-1996 (from Chapman et al., 1997a).

It is believed that spikes of contamination from cattle which are shedding high levels of a specific *E. coli* O157: H7 subtype in their faeces may be responsible for contaminating relatively large lots of products and causing disease because human outbreaks have been associated with single subtypes (Gannon, 1999).

3.1.3 Foods

E. coli O157:H7 has been isolated from beef, beef products, milk and milk products (Table 2), and these products are implicated as sources of human infection. Besides, *E. coli* O157: H7 have also been isolated from lamb and lamb products and is implicated as a potential vehicle of infection to humans (Chapman et al., 2000). A wide variety of food items, mainly vegetables or fruits contaminated indirectly with animal manure have also been identified as sources of human infection (Chapman et al., 1997b). The Centre for Disease Control and Prevention of the United States have listed followings foods as found to be vehicle of *E. coli* O157:H7 infection: beef (roast, hamburger, ground beef, beef steak), venison jerky, pizza, sandwich, tacos, apple cider, milk, chicken soup, potato salad, salami, lettuce leaf, and ice/water (CDC, 2002). Cheese and yoghurt were also implicated in outbreaks of EHEC infections (Ammon, 1997). Home-made hamburgers were particularly implicated as the risk for *E. coli* O157: H7 infection as they are typically prepared just before or at the same time as the rest of the meal- providing an

opportunity to cross-contaminate with other meal items (Mead et al., 1997, Russell et al., 2000).

Table 2. Prevalence rate of *E. coli* O157:H7 in different food-items throughout the world

Food	Country	Year	Prevalence	Reference
Beef products (mince/sausages)	United Kingdom	1996-1997	1.1% (n=3216)	Chapman et al., 2000
Lamb Products (mince/sausage)	United Kingdom	1996-1997	2.9% (n=1020)	Chapman et al., 2000
Minced beef and pork	Switzerland	2001	1.75% (n=400)	Fantelli and Stephan, 2001
Minced beef	Argentina	2001	3.8% (n=160)	Chinen et al., 2001
Fresh sausages	Argentina	2001	4.8% (n=83)	Chinen et al., 2001
Minced beef	France	2002	0.12% (n=3450)	Vernozy-Rozand et al., 2002
Minced beef, beef burgers	Republic of Ireland	2004	2.8% (n=1533)	Cagney et al., 2004

As with the prevalence of *E. coli* O157: H7 from cattle faces, similar seasonal trend was observed in isolation of this organism in raw beef and lamb products (Chapman et al., 2000). Highest isolation rate (4%) was reported during the month of July, whereas the lowest (0.2%) during the month of December and February (figure 2). However, no seasonality was found in minced beef samples in a French study (Vernozy-Rozand et al., 2002).

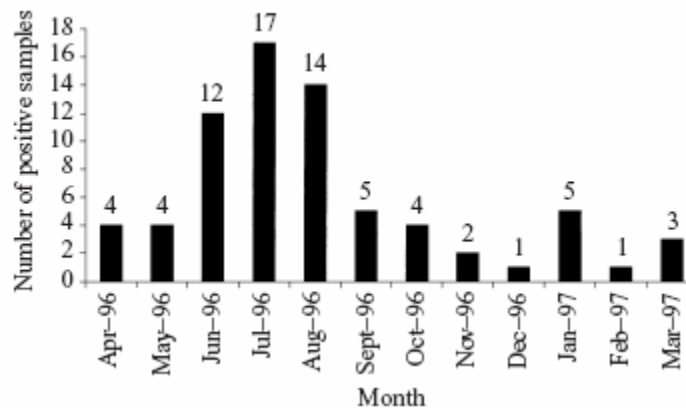


Figure 2. Seasonal prevalence of *E. coli* O157 in raw beef and lamb products (from Chapman et al., 2000).

3.1.4 Environment

VTEC is generally considered to be ubiquitous in the environment (Reinders et al., 2001). Cattle farm environment is particularly implicated to be reservoir of *E. coli* O157: H7 (figure 3) since the organism can persist on the cattle farms for years (Hancock et al., 2001). Besides, farms rearing other species of animal along with cattle may also harbour the organism in the farm environment. In one Swedish study, *E. coli* O157: H7 was found to persist in a herd of pigs in a farm environment for a period of up to 11 months (Eriksson et al., 2003). Animal beddings, water troughs and manure slurry are reported to harbour *E. coli* O157 (Conedera et al., 2001).

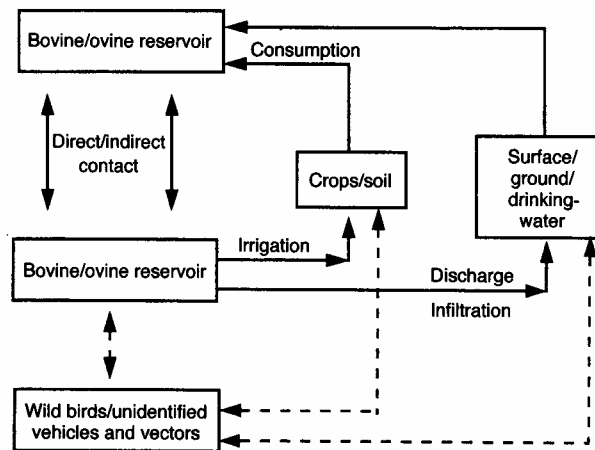


Figure 3. The ecological cycle of *E. coli* O157: H7 in the farm environment (Wallace, 1999)

E. coli O157: H7 survives well in bovine faeces, and the growth is dependent on temperature and water activity (Wang et al., 1996). Tolerance to extremes of temperatures (+4°C to -20°C) was exhibited by the organism in the bovine faeces (Bolton et al., 1999). Wang et al. (1996) reported that *E. coli* O157:H7 survived for up to 70 days in bovine faeces at 5°C, which was reduced to 56 days at 22°C and 49 days at 37°C. Shorter survival days at higher temperatures are believed to be due to the reduced water activity associated with drying of faeces (Wang et al., 1996).

The number of the organism in the environment is also dependant on the period under consideration. During 99 days, the count of *E. coli* O157: H7 in a grassland experimentally contaminated with cattle faeces was found to decline from 7.85 log₁₀ CFU per g to 2.96 log₁₀ CFU per g faecal sample (Bolton et al., 1999). Follow-up of this

pasture land revealed that *E. coli* O157: H7 was still present in 75% of the surrounding soil samples after 85 days, and in 20% of the samples after 99 days of contamination.

3.2 The illness

3.2.1 Transmission and risk factors

All the pathogenic *E. coli* are transmitted via the faecal-oral route. The pathogen can be transmitted by direct or indirect contamination of foods by faecal material. Since the gastrointestinal tract of cattle is an identified reservoir of this pathogen (Chapman et al., 1997a), contamination of food by bovine faeces is a likely route of transmitting *E. coli* O157: H7 to humans. Person-to-person transmission has also been documented as secondary spread after foodborne infection (Chapman et al., 1997b; Anon, 2000).

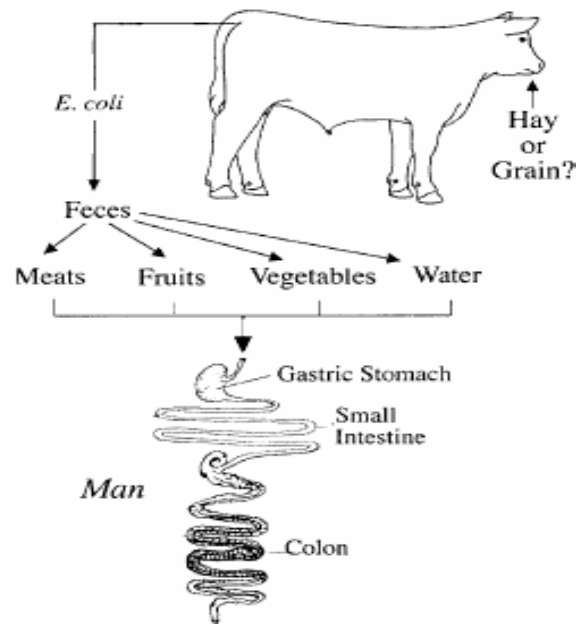


Figure 4. A scheme showing possible routes of transmission of *E. coli* from cattle to human (from Russell et al., 2000)

Mead et al. (1997) documented the following risk factors in a case-control study.

- Failure of food handlers to wash hands with soap and water, consistently after handling ground beef ,
- Not washing work surfaces that had been in contact with ground beef,

- Inadequate kitchen hygiene like placing cooked hamburgers back onto an unwashed plate that had previously held raw ground beef,
- Serving undercooked ground beef,
- Leaving raw ground beef unrefrigerated for more than 2 hours,
- Tasting small bits of raw ground beef during meal preparation,
- Cross-contamination of other meal items or utensils.

Besides, consumption of raw milk is also considered to be an important risk factor. Other risk factors include extremes of age (<4 and >70 years), leukocytosis, and use of antimotility agents and antibiotics for the bacterial enterocolitis (Wong et al., 2000).

3.2.2 Symptoms

Haemorrhagic colitis (HC) is the principal illness caused by *E. coli* O157: H7, diarrhoea being the most common clinical presentation, with an incubation period of 1-14 days, which is frequently, but not invariably, bloody in nature, commonly accompanied by severe, cramping abdominal pain. Vomiting occurs in about half of patients, but fever is uncommon, and the illness resolves in 5-10 days (Eslava et al., 2003). In some cases, the diarrhoea symptoms may be very severe, consisting of all blood and occurring in every 15-30 minutes. This severe haemorrhagic colitis (HC) may be seen in the very young or old patients (Coia, 1998). However, some infections may also be completely asymptomatic (Eslava et al., 2003).

Haemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) are the most important complications of *E. coli* O157 infection (Elliott and Nichols, 2001) which may develop in up to 11% of the affected individuals (Dundas et al., 1999). HUS is particularly common in the children under 5 years of age and the elderly (Eslava et al., 2003). TTP is further complication of HUS, characterized by the development of neurological and other and other organ involvement, and is seen most often in adults (Coia, 1998). HUS and TTP are multisystemic disorders characterized by thrombocytopenia, microangiopathic haemolytic anemia, and nephropathy and organ ischemia (Dundas et al., 1999).

3.2.3 Pathogenesis and consequences

As Shiga-like toxins elaborated by the *E. coli* are absorbed into the systemic circulation, HUS and TTP-like syndrome are produced. These toxins bind preferentially to specific glycolipid receptors on vascular endothelial cells, inhibit protein synthesis causing cell death, leading to endothelial injury and localized intravascular thrombosis. The vascular endothelium of kidney is specifically susceptible, so renal failure results in the infected individuals.

Injury to the renal endothelium is probably the primary etiological event in HUS. The lumen of glomerular capillaries is narrowed as a result of swelling of the injured endothelial cells, hypertrophy of mesangial cells and detachment from the underlying basement membrane. Further occlusion of the narrowed glomerular capillaries and afferent arterioles occurs with the formation of platelet thrombi and fibrin generation and ischemic glomerular and tubular necrosis leads to renal injury. Similar, although pathologically different, events occur in TTP, the other thrombotic microangiopathy which may occur following VTEC O157 infection.

The onset of HUS/TTP occurs very fast, usually 2 to 14 days after the onset of diarrhoea, and by the time the syndrome manifests, diarrhoea may have resolved completely (Elliott and Nichols, 2001).

In children, these microangiopathic syndromes are often self-limited and resolve with supportive care, which may include temporary haemodialysis. However, antibiotic treatment of children with *E. coli* O157:H7 infection has been found to increase the risk of HUS (Wong et al., 2000). Mortality rate is low (3-5%) in children but high in extremes of age, particularly elderly (Dundas et al., 1999). The most important sequelae is chronic renal insufficiency in a significant percentage of affected children (Elliott and Nichols, 2001). Besides, other vascular territories may also be affected, causing ischemic symptoms and neurologic manifestations. In both HUS and TTP, other infrequent consequences are encephalopathy, cardiomyopathy and *diabetes mellitus* resulting from involvement of the brain, myocardium and pancreas, respectively (Coia, 1998).

3.3 Incidence and outbreaks

In the United States, *E. coli* O157: H7 causes over 73,000 illness each year and approximately 61 deaths (Mead et al., 1999). The hospitalization rate and case-fatality rate is respectively 0.295 and 0.0083 (Mead et al., 1999). In Europe, wide variation was seen in incidence rates between countries (Table 3). Lowest incidence rate of 0.1 per million was reported in Spain, while highest incidence rate of 20.3 per million in the United Kingdom. As with isolation rate in cattle and ground beef, seasonal trend is also observed in human infections, with more outbreaks during the summer months (Chapman et al., 2000).

Table 3. Number of reported cases of EHEC infections in Europe, 1996 (from Ammon, 1997).

Country	EHEC infections	Per million inhabitants
Spain	4	0.1
Italy	9	0.2
Netherlands	10	0.6
Finland	5	1.0
Denmark	6	1.2
Austria	11	1.4
Germany	314	3.9
Belgium	52	5.2
Sweden	118	13.6
United Kingdom	1180	20.3

Outbreaks of infection, generally associated with beef, have been reported in Australia, Canada, Japan, United States, in various European countries, and in southern Africa. In 1996, an outbreak of *E. coli* O157:H7 in Japan affected over 6,300 school children and resulted in 2 deaths. This is the largest outbreak ever recorded for this pathogen (WHO, 2002). Similarly, in 1999 in the United States, *E. coli* O157 outbreaks were reported from 30 states and affected 1897 persons. Two hundred one (11%) persons were hospitalized, 37 (2%) developed HUS and 4 (0.2%) died (CDC, 1999).

4 Control

As bovine reservoir is believed to be of major importance to the occurrence of VTEC infections in humans, reduction of this organism in cattle is the most effective means to control its spread through meat, milk or other transmission routes. Control has been challenged in the food industry, particularly beef industry, by the very low infective dose and ability of the organism to withstand low pH (Booth et al., 1999).

To make the effective implementation of the control measures, they may be categorized as short-, middle-, and long term priorities (Reinders et al., 2001). Short-term priorities will deal with the matters of immediate concern like the outbreak situations. Mid-term priorities will include surveillance of the organism in the farm, the animals and the food. Likewise, long-term measures will include the integration and application of the finding of the above surveillance for the formulation of effective control measures like implementation of hygienic farm management and feeding practices.

It is generally felt that control strategies for this and other foodborne pathogens must extend along the food-production continuum (from 'farm to fork') and include not only slaughter and processing steps but also measures to control the pathogen in cattle populations. At the other end of this continuum, steps can be taken to limit the growth and survival of *E. coli* O157:H7 during transport, storage and retail display and to provide effective decontamination and cooking procedures.

4.1 On farm control

Hygienic post-slaughter processing practices effectively reduce carcass contamination with *E. coli* O157: H7, but on farm (pre-slaughter) intervention strategies offer the opportunities to reduce the microbial load in animals before they enter the food chain. Bovine faeces is a potential source for spreading *E. coli* O157: H7 to human food chain as well as to the environment. Contamination of the environment and food by *E. coli* O157:H7 can be prevented through effective control of carriage of this organism in cattle and proper handling or usage of the manure from these animals (Wang et al., 1996). It has been suggested that pre-slaughter reduction of *E. coli* O157: H7 prevalence in cattle would result in considerable reduction of contamination of beef and consequent human disease (Jordan et al., 1999).

4.1.1 Feed and water hygiene

Cattle and other farm animals are exposed to *E. coli* and other faecal organisms, most commonly via feed and water that might have contaminated with these organisms. A wide variation in the level of contamination of feed and water has been found suggesting this level is potentially subjected to management practices of the farm (Hancock et al., 2001). Feed might have been contaminated before purchase or during on-farm storage or mixing. Purchased feed are also implicated as primary vehicle for regional dissemination of *E. coli* O157: H7. One of the reasons for increased faecal shedding of *E. coli* O157: H7 from animals during summer months may be increased *E. coli* counts in trough water, and growth of the organism supported by wet grain mixes, during this time period (Hancock et al., 2001). Theoretically, it appears that implementation of strict feed hygiene practice is the solution, but is difficult on practical grounds. Water-borne infections may be avoided by frequent cleaning of the water troughs to prevent the persistence of *E. coli* O157 in water (Conedera et al., 2001).

4.1.2 Reducing faecal shedding

Competitive inhibition: Probiotics are the bacteria that are administered orally for the competitive inhibition of target an organism. It has been practiced widely in monogastric animals, mainly in poultry production. Limited information is available regarding the ruminants. It has been suggested that administration of bovine commensal *E. coli* that colonize that same anatomic locations as *E. coli* O157: H7 and produce toxic metabolites like colicin may reduce the carriage of *E. coli* O157 in the bovine digestive tract (Harmon et al., 1999). Zhao et al. (1998) demonstrated that inoculation of cattle with non-toxicogenic *E. coli* and *Proteus mirabilis* can reduce the level of carriage and faecal shedding of *E. coli* O157: H7 in most animals. Another advantage of administering probiotics in ruminants appears to be their competition with *E. coli* O157: H7 in other locations within the farm environment, such as in the feedlot manure (Harmon et al., 1999). These findings are very promising since even if such probiotic preparation reduces only 50% of the faecal shedding of the *E. coli* O157: H7 that would have a marked influence on ecology of this organism which will in turn reduce the level of environmental exposure to other animals (Hancock et al., 2001).

Another way of inhibiting the survival of *E. coli* in rumen is by creating an unfavourable environment, e.g. through elevating the VFA level, either by dietary supplementation of VFAs or stimulating the VFA production from commensal anaerobes (Duncan et al., 1999). It has been suggested that ruminal and intestinal VFA concentrations limit the proliferation of *E. coli* (Wolin, cited by Callaway et al., 2003).

Pre-slaughter feeding management: Cattle feeding practices may be manipulated to decrease the gut proliferation and faecal shedding of *E. coli*. The extent of carcass contamination is believed to be dependent on the number of *E. coli* O157:H7 shed per gram of faeces at slaughter (Gannon, 1999). As *E. coli* shedding in faeces is dependent on the ruminal count of the organism, manipulating its survival in the rumen before transport for slaughter appears to be an effective way of reducing the counts of *E. coli* in the faeces (Gregory et al., 2000). Faecal shedding is believed to be facilitated by survival of the passage of the organism through the acidic abomasum and subsequent localization in the large intestine (Brown et al., 1997). When cattle are fed high grain-based rations, some starch reaches the hindgut escaping the ruminal digestion, which is believed to facilitate the multiplication of *E. coli* there (Callaway et al., 2003). This also increases the acid-resistance of the organism which will ultimately facilitate its survival on the low pH environment of human stomach (Russell et al., 2000). It has been widely accepted that abrupt switching of cattle from a high grain-ration to high-quality hay-based diet reduces the generic *E. coli* and *E. coli* O157: H7 population, also their acid tolerance (Russell et al., 2000, Hancock et al., 2001; Callaway et al., 2003). This method is believed to be having widespread preslaughter application in the cattle industry, also because the effect is almost immediate (Russell et al., 2000).

Fasting cattle results in proliferation of coliforms in the bovine rumen and subsequent increase in faecal shedding (Harmon et al., 1999; Brown et al., 1997; Gregory et al., 2000). As feed withdrawal or fasting results in lower VFA concentration in the rumen and hindgut (Callaway et al., 2003), there will be no longer the inhibitory effect of VFA on proliferation of *E. coli*. So, long distance transport of cattle before slaughter for up to 48 hours, without food, has been shown to increase *E. coli* populations throughout the intestinal tract (Gregory et al., 2000). Feeding of hay or other roughages 48 hours before dispatch for slaughter may be helpful in decreasing this bacterial proliferation (Gregory et

al., 2000). This practice was also found to keep the soiling of hides to a minimum (Gregory et al., 2000).

Feeding of limestone to cattle may also reduce the incidence of acid tolerance in *E. coli* (Scott et al. cited by Russell et al., 2000).

It has been suggested that if ruminal fermentation of grains can be enhanced in such a way that minimal unfermented starch reaches the colon, proliferation of *E. coli* may be prevented there. Russell et al. (2000) mentioned that it may be achieved by processing the grains, e.g. by heating, so that protein coating of the starch granules breaks-down and the starch becomes readily available for ruminal fermentation.

Vaccination: Several groups are working on *E. coli* O157: H7 vaccines for cattle, but none have been successful for a breakthrough. As with competitive inhibition, even a slight reduction in faecal shedding of the organism would have substantial impact on the dynamics of *E. coli* O157: H7 in a farm ecosystem (Hancock et al., 2001). Jordan et al. (1999) in a simulation study suggested that vaccination has the greatest potential impact to reduce the shedding of *E. coli* O157 in faeces.

4.1.3 Reductions in hide soiling

As hide is implicated to be an important source of bacterial contamination of carcasses, including *E. coli* O157 (Gannon, 1999), efforts to reduce the faecal soiling of body coat seems to be important for control of this organism. Taking this fact into consideration, it has been reported that some processing plants in the USA have started to discriminate between clean and heavily soiled animals (Hancock et al., 1999). However, Jordan et al. (1999) suggested that reduction of visible hide soiling would have very small impact on reducing the *E. coli* O157 contamination of carcasses. Likewise, Byrne et al. (2000) also documented that pre-slaughter hide washing for 3 minutes did not significantly reduce the subsequent contamination of carcass.

4.1.4 Manure recycling practices

Bovine faeces or contaminated pastureland supports the survival of *E. coli* O157: H7 for extended periods, up to several months (Bolton et al., 1999). Contamination of pastureland with *E. coli* O157: H7 may be the result of farm waste recycling practices

like the spreading of manure onto it or irrigating the pasture with cow manure slurry. This in turn may result in increased carriage of the organism in bovine herds (Wang et al., 1996), posing great risks to the final consumer of resulting milk and meat products (Bolton et al., 1999). So, proper management and/or treatment of manure and slurry may also be important in preventing contamination of farm environment and infection in cattle.

4.1.5 Surveillance of the organism

These measures include surveillance of the organism in animal population on farm, after transportation (i.e. shedding of VTEC, and the spread of the organism between herds or among animals entering slaughter facilities) and population dynamics in dairy farm (i.e. duration of shedding by individual animals, and the extent of on-farm VTEC transmission among animals) as well as studies of farm environment. Identification of *E. coli* O157: H7 positive cattle before slaughter is a crucial step in any on-farm control measures to reduce the risk of carcass contamination with this pathogen. In one Canadian report (Anon, 2000), it was recommended that slaughtering of cattle, shedding more than 10^4 CFU/g of faeces of *E. coli* O157: H7, should be done separately.

4.1.6 Quarantine and farm hygiene

If a farm is suspected of harbouring high-risk infective materials, the infection should be restricted and further spread of the infection from the farm should be prevented. It may be achieved by imposing strict quarantine measures. The imposition of quarantine involves the restriction of movement of animals and animal products (with the exception of milk) as long as a farm is considered positive. Milk from such farms should be pasteurized before removal. Manure should be stockpiled, and if necessary, treated. Before spreading on land, it should be made sure by bacteriological analysis that the manure is free from the organism.

Contaminated materials within the farm should be restricted in a system which prevents proliferation of the organism and prevent animal-to-animal transmission. This can be achieved by a range of hygienic measures, including separate rearing of animals of different species (Eriksson et al., 2003) or age groups (Reinders et al., 2001), regular

cleaning and disinfection of troughs and bowls, quick removal of feed leftovers and strict personal hygiene among farm workers. All animals on the farm should be sampled every 2 weeks, as regular and repeated bacteriological examination is necessary to accurately determine that each animal is truly free from *E. coli* O157, rather than merely irregularly shedding the pathogen. In general, a farm may be considered free of *E. coli* O157 when all samples are negative for the organism over two successive samplings. Animals that are shedding the organism for more than 6 weeks or over three successive samplings are considered to be long-term shedders, and should be removed (Reinders et al., 2001).

However, implementations of hygienic on-farm control measure are difficult, particularly in the types of farming where different species and age groups of animals co-exist in contagious pens (Conedera et al., 2001).

4.1.7 Eradication of the organism

The level of infection in a herd with the strains of VTEC O157 is very dynamic. Some strains are present for long periods, while others are less persistent. Hence a farm may soon acquire new strains of the organism after completion of an eradication program. Regardless of the potentials of reinfection, eradication programmes should be carried out after a herd has been exposed to an outbreak (Reinders et al., 2001). However, eradication seems to be unsuccessful for such a ubiquitous organism that is not host specific (Hancock et al., 2001).

4.2 Control at slaughter

It has been suggested that *E. coli* contaminates beef carcass in the processing plants either directly from spillage of ingesta and faecal materials onto the carcass during processing or through faecal material that is transferred from the hide, or by contact with workers or equipments that have been contaminated. As *E. coli* O157: H7 has been reported in faeces, rumen contents and on the hide of cattle at slaughter (Brown et al., 1997; Chapman et al., 1997a, Wang et al., 1996), prevention of possible contamination of carcass is the most significant challenge to the meat processing plants. This is largely dependant on the *E. coli* O157: H7 status of the preslaughter animal, and the processing techniques which distribute the organism within or between carcasses during dressing

operations. Although it is not possible to completely avoid carcass contamination during slaughter and processing, it can be minimized to a very low level by strict hygienic measures like the hazard analysis critical control points (HACCP) (Gannon, 1999).

4.2.1 Controlling sources of carcass contamination

The most important sources of contamination of carcasses following slaughter are associated with skinning and bung tying (McEvoy et al., 2003), and include: faeces, hide soils, aerosols and sprays, contact with workers' hands, gloves and other equipment and accidental spillage of body fluids during evisceration. Contamination of carcass can take a number of forms (Gannon, 1999).

1. Accidental, random, visible contamination of carcasses, e.g. when faeces or intestinal contents contaminate a carcass following a knife puncture.
2. Systematic visible contamination, e.g. defined areas or zones of the carcass shown to have higher visible demerit scores than others.
3. Accidental or random invisible contamination from aerosols, dusts and sprays.
4. Systematic invisible contamination from contact, smears, aerosols, dusts and sprays.

It has been reported that most of the microbial contamination of the carcass in the processing line occurs during skinning of the hindquarters (Gill et al., 1998). As there is likelihood of these organisms coming directly from faeces in the anal region, rump region is the most vulnerable for contamination with *E. coli* (Gill et al., 1998). However, this can be minimized by enclosing the anus and adjacent portion of the rectum or 'bung' in a plastic bag and binding this to the outer wall of the rectum (Gannon, 1999; Russell et al., 2000). Bell (1997) mentioned that the neck, the brisket, legs and hooves are also the areas that are highly prone to contamination with *E. coli*. This is presumably related to contact between these areas of carcass and the outside surface of the hide during skinning (Gannon, 1999; McEvoy et al., 2003).

Preslaughter washing: Preslaughter washing of the animals may be an appropriate technique of decontaminating animal hides, and preventing the subsequent carcass contamination. Byrne et al. (2000) reported that preslaughter washing of bovines for 3

minutes significantly reduced hide faecal contamination with *E. coli* O157: H7, however, did not significantly result in lower carcass contamination.

4.2.2 Cleaning contaminated carcasses

If the contamination area is less than 2.5 cm in its greatest dimension, it should be removed either by **knife trimming** or by **steam vacuum**, and if larger than 2.5, should be trimmed away with a knife (Gannon, 1999). Visible contamination may also be reduced by **washing** the carcass simply with cold or warm water. Though not an effective method to eliminate all the organisms, cold water wash may decrease the microbial contamination following skinning by reducing the ability of the organism to adhere to the carcass surface. Hot water treatment (discussed later) is, however, believed to be a more promising method to decrease the risk of *E. coli* O157: H7 contamination of beef carcasses which consists of a combined whole-carcass treatment. However, these procedures alone are not reliable enough to ensure the elimination of the organism from the carcass and safety of the product. This is partly, because of the problem of standardizing these methods, and more importantly, because much of microbial contamination is invisible. “Zero-tolerance” to visible contamination of carcass should be set as a requirement by legislation to promote spot-clean of visible areas of contamination of carcass during processing.

4.2.3 Decontamination

Several methods to reduce contamination of carcasses have been developed and tested for efficacy against *E. coli* O157:H7. These methods, which include the use of hot-water washes, steam vacuum, and chemical treatments, either alone or in combination, have various degrees of success in reducing or eliminating this pathogen.

Using hot water and steam: Heat treatment, either in the form of hot-water washes or pressurized steam, is a simple way of whole-carcass decontamination to decrease high levels of the pathogen at all points on the carcass surface. The aim of heat treatment is to raise the temperature of carcass surface sufficiently high to kill *E. coli* O157: H7, along with other pathogens. One drawback of this technique is change of color due to surface heating, which may be unacceptable to consumers. However, this effect is a temporary

and the carcass returns to normal color, if the duration of treatment is short (Gannon, 1999).

Chemical treatments: The level of contamination of carcass may be reduced effectively by washing it with a variety of disinfectants added to the wash-water. Examples of some such disinfectants are hydrogen peroxide (5%), trisodium phosphate (8-12%), acetic acid (2%) ozone (0.5%), fumaric acid (1%), lactic acid and acetic acid (Gannon et al., 1999). It was observed that temperature and number of wash-water treatments were the most important factors determining the reduction of number of organisms. Two washes, with the temperature of first wash-water 72°C, was found to be comparatively more effective (Gorman et al., cited in Gannon et al., 1999). Trisodium phosphate (TSP) may be used at 8% concentration (which has a pH of >12) as a postchill antimicrobial treatment as it is found to be effective in reducing *E. coli* O157: H7 in beef carcasses and potentially other food items (Feng, 2001).

Combined treatments: It was mentioned that when carcass cleaning and carcass decontamination methods were used in combination, the techniques were found to be more effective in eliminating *E. coli* O157: H7 from carcass, as compared to any one treatment alone. The following combinations were found to be equally effective in reducing *E. coli* O157: H7 levels from beef carcasses (Phebus et al., cited in Gannon, 1999): (i) trimming and a warm-water wash; (ii) trimming, a warm-water wash and steam pasteurization; (iii) a warm-water wash and steam pasteurization; (iv) trimming, a warm-water wash, a hot lactic acid wash and steam pasteurization; and (v) a steam vacuum, a warm-water wash, a hot lactic acid wash and steam pasteurization. Farkas (1998) documented that hot water or pressurized steam, when used in combination with hot lactic acid or acetic acid solution, is an effective way of decreasing the level of *E. coli* O157: H7 contamination of beef carcass.

4.2.4 Irradiation

Ionizing radiation is mentioned to be the most effective method in eliminating *E. coli* O157: H7 from ground beef (Gannon, 1999). It has been reported that a dose range of 2-3 kGy of gamma irradiation is adequate to decontaminate raw meat of all food borne pathogen including *E. coli* O157: H7 (Feng, 2001). One of the advantages of irradiation

is that it can be applied in prepackaged meat products, and those that are frozen (Farkas et al., 1998), so recontamination of the product is also less of concern. The dosage requirement, however, depends on the temperature of the product during irradiation (e.g. fresh, refrigerated, or frozen), in addition to the physiological state of the organism (i.e., induced to acid resistance state).

However, there are certain limitations of this method, like changes in sensory attributes of meat, regulatory approval only in limited countries, and cost-effectiveness of industrial application.

4.2.5 Freezing

The conditions of freezing and thawing (rates, method, temperature achieved, etc.) affect the extent of damage and death to the cells of *E. coli*, but it is clear that survival of freezing process can and does occur. Dependence on freezing to decontaminate food is therefore not a reliable practice (Bell and Kyriakides, 1998). A study done by Dykes et al. (1999) to assess the survival of *E. coli* O157:H7 on frozen beef trimmings found that freezing in itself is not a reliable method of product decontamination, however, it may add margin of safety to beef trimmings contaminated with *E. coli* O157:H7, if used in combination with other methods.

4.3 Protection of Consumers

4.3.1 Proper cooking

Cooking is the safest methods of avoiding food-borne bacterial infections, including *E. coli* O157 (Gannon, 1999). Cooking is of especial importance in preventing *E. coli* O157:H7 infection as most outbreaks are related to consumption of undercooked ground beef. It is thus obvious that all ground beef and hamburger should be cooked thoroughly. Because ground beef can turn brown before *E. coli* are killed, to ensure thorough cooking digital instant-read meat thermometers can be used. Ground beef should be cooked until a thermometer inserted into several parts of the patty, including the thickest part, reads at least 160°F. It is suggested that persons who cook ground beef without using a thermometer can decrease their risk of infection avoiding eating ground beef patties that are still pink in the middle.

4.3.2 Kitchen and personal hygiene

Cross-contamination from contaminated meat to other foods is one of the most significant ways of getting infected from *E. coli* O157. To avoid this, raw meat should be kept separate from ready-to-eat foods. Hands, counters and utensils should be washed with hot soap water as they touch raw meat. Cooked hamburgers or ground beef should never be placed on the unwashed plates that held raw patties. Meat thermometers should be washed in between test of patties that require further cooking.

Persons with diarrhoeal illness should be taken as special risks and should not be preparing meals, serving foods, or handling clean plates and utensils that are used in serving, and their washing of hands with soap and water is crucial before they touch food items or utensils.

4.3.3 Change in Food-habit

Only pasteurized milk, juice or cider should be consumed. Fruits and vegetables, particularly those that are eaten raw, should be washed thoroughly. Proper washing and rinsing of the produce prior to consumption may not eliminate the risk completely but have been shown to significantly decrease the bacterial populations on the produce (Feng, 2001). Children, elderly and immunocompromised people should not be allowed to eat high risk food like alfalfa sprouts, until their safety can be guaranteed. Water that has been treated with chlorine or other disinfectants should be drunk.

4.3.4 Educating of the public

As substantial proportion of *E. coli* O157: H7 infections are acquired in the home as a result of inadequate kitchen hygiene, it has been suggested that these infections could be prevented through enhanced kitchen hygiene and safe food-handling at home (Mead et al., 1997). Introduction of safe food-handling instruction label (figure 4) on the meat packages is believed to be one way of improving these food-handling practices.

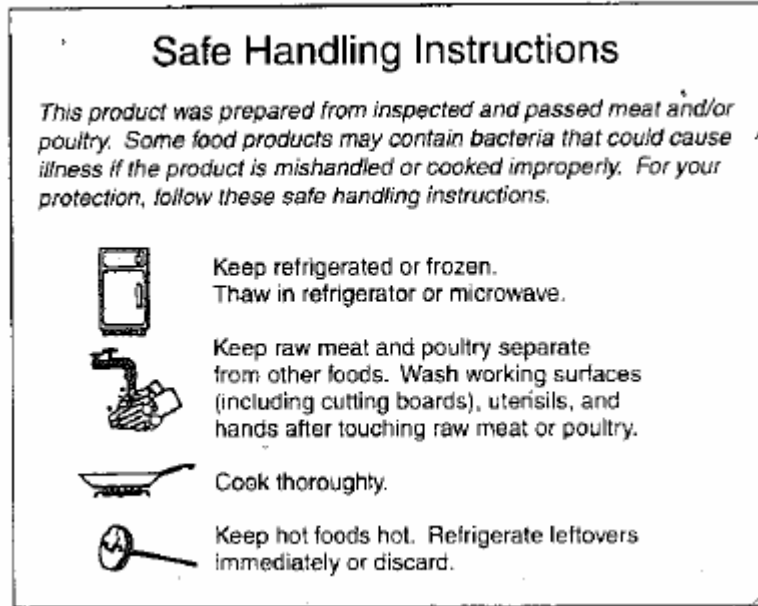


Figure 4. Safe food-handling procedures required on all meat packages (from Mead et al., 1997).

Since it is impossible to make the meat completely free of pathogens, improvement in consumers behavior remains the important means of preventing *E. coli* O157: H7 infections (Mead et al., 1997). As *E. coli* O157: H7 present in food is rapidly killed on cooking (Chapman et al., 2000), consumers should be made aware about this, e.g. by the safe food-handling label.

Consumers should be educated about the risk of eating undercooked ground beef, the importance of safe food-handling practices, and the potential for person-to-person transmission.

Food handlers infected with *E. coli* O157 should not be allowed to prepare food until they are asymptomatic and have had two negative stool cultures (CDC, 1995).

5 Conclusions

In spite of being a relatively uncommon pathogen, *E. coli* O157: H7 and other EHEC may be quite common in animals and meat, and a considerable effort will be required at all sectors of the food production, processing, service industries, and by the consumer to reduce the incidence of HC and other diseases caused by these organisms.

Although at the present moment, on-farm control options do not appear to be very effective, scientific progress in such areas as the development of probiotics, improved

feed hygiene, biosecurity and vaccination may, in longer term, contribute to the elimination of VTEC from livestock and meat. However, these options are not for immediate future application as they have not been fully researched or sufficiently developed. For the present moment, application of hazard analysis critical control point (HACCP) principles and good hygienic processing practices at the slaughter houses seems to be most significant option of controlling *E. coli* O157: H7 infection in humans. HACCP should be implemented even more stringently in the context of modern complex processing and widespread distribution of foods. As it seems that with the existing processing techniques production beef completely free of *E. coli* O157: H7 is unlikely, consumer's protection may only be achieved by their education on proper food handling. Surveillance of EHEC related diseases is important to understand the epidemiology of the infection in humans and to devise an effective control program.

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