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Approach to the Control of Entero-haemorrhagic *Escherichia coli* (EHEC)

Report

Prepared under the responsibility of the
ILSI Europe Emerging Pathogen Task Force

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Report on the Approach to the Control of Entero-haemorrhagic *Escherichia coli* (EHEC).

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APPROACH TO THE CONTROL OF ENTERO-HAEMORRHAGIC ESCHERICHIA COLI *(EHEC)*

REPORT

PREPARED UNDER THE RESPONSIBILITY OF THE ILSI EUROPE EMERGING PATHOGEN TASK FORCE

MAY 2001

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FOREWORD

Entero-haemorrhagic *Escherichia coli* (EHEC) are, collectively, one of the greatest microbiological challenges to hit the food industry since the scourge of botulism some 80 years ago. EHEC are bacteria that are a challenge because they:

- are highly infectious in humans,
- have a very low infectious dose,
- cause serious acute illness,
- cause serious long-term sequelae, especially kidney failure,
- are naturally occurring in cattle (and other animals) and, hence, are in the soil,
- are not clinically apparent in infected cattle (and other animals), and
- occur globally.

The challenge to the food industry posed by EHEC today is far greater than the challenge posed years ago by botulism. Although effective processing in food production facilities can control both *Clostridium botulinum* and EHEC, their presence on fresh produce intended to be eaten raw has very different consequences for public health. A few spores of *C. botulinum* on fresh produce will not cause harm to consumers, whereas a few cells of EHEC may cause serious illness, especially in children.

Thus, the real problem posed by EHEC cannot be solved simply in the food manufacturing plant, catering service, or the home. It has to be solved on the farms where raw food materials are raised or grown. The ecology of EHEC is not yet fully understood so their control is likely to be far more complex than the rules to control *C. botulinum* in the food chain. Interventions are also required at all subsequent steps in the food chain.

Difficulties and confusions have evolved with the nomenclature of entero-haemorrhagic *E. coli.* Strains of *E. coli* that produce potent cytotoxins were first reported in 1977. They were described as verotoxin-producing *E. coli* (VTEC), because the cytotoxins are active on vero cells in tissue culture. It was then discovered that the toxins are similar to Shiga toxins, and so the organisms became known as Shiga-like toxin-producing *E. coli* (STEC). STEC contain subgroups of *E. coli* with many different properties and virulence factors. One subgroup of STEC is EHEC. These organisms have been linked to a spectrum of disorders including watery diarrhoea, bloody diarrhoea (haemorrhagic colitis), and haemolytic uraemic syndrome (HUS) as described in this report. Generally speaking, all EHECs are STECs, but not all STECs are EHECs. The predominant pathogenic EHEC in the world is *E. coli* O157:H7, and it is the main subject of this report. Further explanation of the nomenclature can be found in the Glossary.

As Chairman of the ILSI Europe Emerging Pathogen Task Force, I warmly acknowledge all of the hard work put into this report by Dr. C. Bell and my colleagues on the task force and its expert group on EHEC.

Dr. John Crowther (Unilever, UK)
Chairman, ILSI Europe Emerging Pathogen Task Force

EXECUTIVE SUMMARY

Entero-haemorrhagic *Escherichia coli* (EHEC) are highly infectious bacterial pathogens in humans.

Outbreaks or incidents of illness are believed to result from very low infective doses of EHEC, e.g. <100 cells.

EHEC cause serious acute illness leading to fatality in some cases and life-long sequelae in other cases.

EHEC are a growing global problem.

EHEC occur naturally in cattle and other food animals, and there is no immediate prospect of eliminating them from the food chain.

Many raw foods that have been directly exposed to animal faecal contamination or indirectly exposed via faecally contaminated water supplies may also be contaminated by EHEC.

EHEC are spread mainly by contaminated foods and water, contact with animals carrying the organisms, and person-to-person contact.

EHEC can be reduced or eliminated from foods by normal, well-managed food production processes, e.g. cleaning procedures and pasteurisation heat processes.

For consistently reliable control of EHEC in the food production chain, it is necessary to

- improve animal husbandry,
- improve human and animal waste and water management,
- improve slaughterhouse practices,
- ensure good agricultural practices and proper application of HACCP-based management and control of food production processes, and
- educate food handlers and the public about their responsibilities for safe and hygienic food-handling practices.

INTRODUCTION AND DEFINITIONS

Escherichia coli has been recognised as an important human pathogen virtually since its discovery in 1885 by Dr. Theodor Escherich during his work on bacteria in infant stools. In more recent years, however, the entero-haemorrhagic *E. coli* (EHEC) have been recognised as a cause of significant, serious illness and even mortality in outbreaks of foodborne illness involving a wide variety of foods.

E. coli is commonly a harmless member of the normal microflora of the intestinal tract of humans and other warm-blooded animals and is acquired by infants within a very few days of birth. However, acquired mobile virulence genes located on so-called pathogenicity islands, on integrated bacteriophages, or on plasmids have conferred different types of pathogenicity to certain strains of *E. coli*. Such strains are classified as entero-pathogenic *E. coli* for infants (EPEC), entero-toxigenic *E. coli* (ETEC), entero-invasive *E. coli* (EIEC), entero-haemorrhagic *E. coli* (EHEC), and entero-aggregative *E. coli* (EAEC) (Nataro and Kaper 1998). Furthermore, distinctive surface structures have been identified which are associated with specific invasiveness or uro-pathogenicity characteristics of the different isolates.

Production of cytotoxins called Shiga toxins is the leading trait of a group of *E. coli* for which three terms have been used concomitantly: Shiga-like toxin-producing *E. coli* (STEC), vero cytotoxin-producing *E. coli* (VTEC), and entero-haemorrhagic *E. coli* (EHEC). Some authors prefer to reserve the term EHEC for those *E. coli* that cause more severe illnesses characterised by bloody diarrhoea or the haemolytic uraemic syndrome (HUS), in contrast to milder, non-bloody diarrhoea caused by VTEC or STEC. However, this differentiation based on clinical severity is not paralleled by the presence of phenotypic or genotypic traits that might be used for assessing the potential risk to humans of isolates from animals, food, or the environment.

To date, at least six virulence factors have been suggested including Shiga toxin production, formation of the adhesion factor intimin, presence of EHEC haemolysin, production of a serin protease and of a heat-stable enterotoxin (EAST), and presence of a special catalase system. Among these, Shiga toxin, intimin, and haemolysin production are considered the most important virulence factors. Some of these factors are encoded on mobile genetic elements, and various combinations of these factors have been identified in isolates from different clinical conditions as well as from non-clinical sources. In the present report, the term EHEC refers to strains producing Shiga toxins either alone or in association with other virulence factors.

Despite this definition, the task force was aware that specific clones belonging to certain *E. coli* serotypes have attained worldwide importance in outbreaks as well as in association with more severe illnesses. Among these, the serotypes O157:H7 / H–, O26:H11 / H–, O103:H2 / H–, O111:H2 / H–, and O145:H28 / H– deserve special mention.

SOURCES AND INCIDENCE OF EHEC

The primary habitat of *E. coli* is the intestinal tract of humans and other warm-blooded animals. *E. coli* infections in humans are transmitted directly from animals, by person-to-person contact and via contaminated foods. Widespread faecal contamination of the environment (soil or water) by farm animals and wild animals provides a continuing source of EHEC in the agricultural environment and hence, in a wide variety of raw foods.

Many serogroups of *E. coli* are found as normal and harmless inhabitants of the mammalian gut, but others are pathogenic to man and animals. Domestic pets such as dogs and cats carry *E. coli* including serogroups containing types pathogenic to humans, e.g. O55, O111, and O128. Other domesticated animals including cattle, pigs, sheep, the young of these animals, and poultry carry *E. coli* as commensal flora, often different from the "normal" and harmless strains in humans. They may also be infected by specific strains different from those infecting humans.

As more research is carried out, many other wild animals and birds are being added to the list of carriers of VTECs pathogenic to humans, and they perpetuate the reservoir of these organisms in the environment and add to the sources of contamination of the raw food supply. However, the EHEC "cycle" in the environment is probably driven primarily by bovine faecal contamination. Faecal shedding of *E. coli* O157:H7 persists longer in calves than in adult cattle, and the type of feed consumed by cattle can influence the prevalence and acid resistance of this organism (Park *et al.,* 1999).

Asymptomatic human carriers are of particular concern in food-handling environments. In one survey, the polymerase chain reaction (PCR) product for verotoxin-encoding genes was detected in 3.5% of more than 5500 stool samples taken from healthy employees in the Swiss meat-processing industry (Stephan *et al.,* 2000). In an earlier report relating to 1730 specimens taken from meat-processing company staff in all parts of Switzerland, Stephan and Untermann (1999) reported that the PCR product was detected in 4.6% of the total samples taken. With regard to employee workplace, the highest positive rate was in slaughterhouse workers, at a level of 9%. Butcher shop and processing workers were less than half this rate, at 4%. Thus, food handlers must be regarded as a potential direct source of EHEC.

Surveys of the incidence of *E. coli* O157 in food animals, their carcasses, and raw meats indicate levels ranging up to 80% (Table 1).

Table 1
Recent reported incidences of E. coli O157 in animal and animal product samples

Subject	Country	Occurrence (%) of STEC	Reference
Retail meat and meat products: survey of fresh and processed meat products from supermarkets and butchers shops over 2 years	Netherlands	O157 STEC (VTEC) isolated from 6/571 (1.1) raw minced beef 2/402 (0.5) raw minced beef and pork mixed 1/76 (1.3) raw minced pork 1/393 (0.3) other raw pork products 1/328 (0.3) cooked or fermented ready-to-eat meats 0/223 (0) other raw beef products 0/819 (0) poultry meat samples 0/46 (0) sheep or lamb samples 0/83 (0) wild animal samples	Heuvelink *et al.*, 1999
Lamb: faeces from lambs in abattoir holding yards	Australia	26/72 (36) STEC 3/72 (4.0) *E. coli* O157:H–	Fegan and Desmarchelier, 1999
Healthy cattle: rectal swabs from healthy animals from 10 dairy farms (n=121) and 4 beef farms (n=60) and 2 samplings from 1 slaughterhouse (n=16)	Brazil	99/121 (82) dairy cattle positive for Shiga toxin gene sequences 40/76 (53) beef cattle positive for Shiga toxin gene sequences 3/197 (1.5) *E. coli* O157:H7 isolated 10 non-O157 Shiga toxin-producing *E. coli* also isolated	Cerqueira *et al.*, 1999
Dairy cattle: faecal samples from 60 herds (average of 41 animals tested per herd); total of 2419 animals tested	Denmark	87/2419 (3.6) cattle positive for *E. coli* O157 10 positive herds in which 3–53% of the animals were excreters (average 21%)	Nielsen, 2000
Cattle: • Slaughterhouse samples (faecal samples from rectum after evisceration) • Swabs from warm carcasses	Denmark	42/195 (22.5) faecal samples positive for *E. coli* O157, 30 (15.5) of which produced verocytotoxin 11/195 (5.6) carcass swabs positive for *E. coli* O157, 3 of which produced verocytotoxin	Boel, 2000

(continued on the next page)

Subject	Country	Occurrence (%) of STEC	Reference
Meat products purchased from small butchers shops: • Raw beef products • Raw lamb products • Mixed meat products	UK	 36 / 3216 (1.1) 29 / 1020 (2.9) 3 / 73 (4.1) lamb sausages 18 / 484 (3.7) lamb burgers 7 / 857 (0.8) All isolates were *E. coli* O157, verotoxin positive and carried the *eae*A gene	Chapman *et al.*, 2000
Faecal swabs from: • Healthy dairy cows • Healthy sheep • Healthy goats	Germany	 131 / 726 (18) STEC 9 / 28 (32.1) STEC 70 / 93 (75.3) STEC No strains were O157 or O111. 1 strain from a bovine source was O26	Zschöck *et al.*, 2000

Cattle are now widely accepted as a major reservoir of *E. coli* O157:H7 (currently the most important of the foodborne EHEC). Potential routes of infection from cattle to humans include:

- faecal–oral route from animals to humans by direct contact with infected animals,
- faecal contamination of food crops when untreated or poorly treated manures are used for fertiliser,
- faecal contamination of water courses subsequently consumed directly or indirectly (via irrigated or washed field crops),
- faecal contamination of carcasses during slaughter and evisceration processes, and
- consumption of faecally contaminated raw milk or raw products derived from it.

Figure 1 gives a simplified overview of the currently understood "cycle" of EHEC in the food-related environment. Significantly, poultry and eggs have not been associated with EHEC in outbreaks of foodborne illness. Also, fish, shellfish, and related products so far have not been implicated in outbreaks of foodborne EHEC. In light of the pivotal role played by water in the cycle, these current gaps in the list of product types implicated in such outbreaks should alert governments, public health officials, and food scientists to the possibility that it may be only a matter of time before these gaps will be "filled".

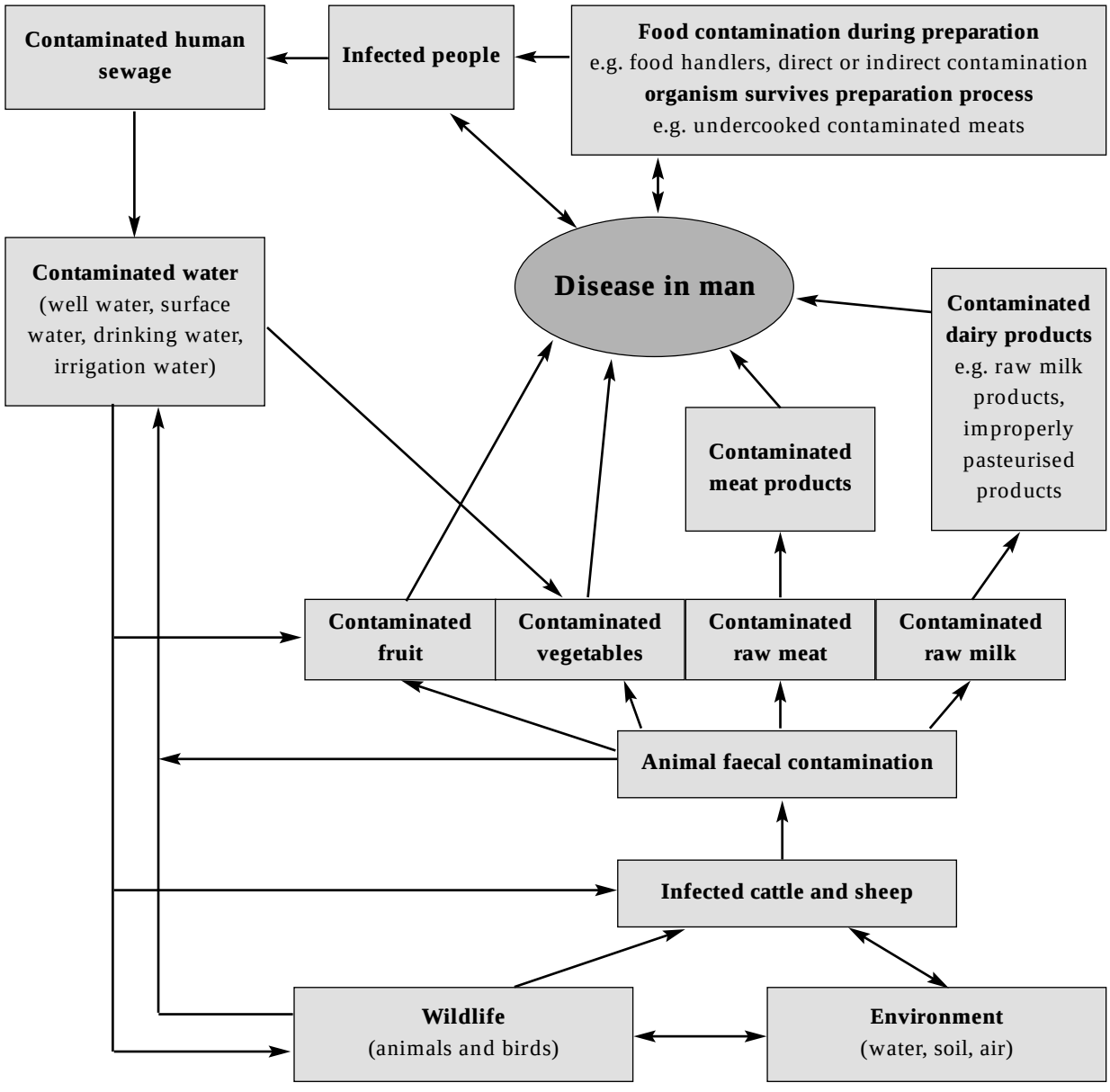
In the European Union, the reported incidence of human cases of EHEC for 1996 ranged from 0.01 per 100,000 population (Spain) to 2.03 per 100,000 population (United Kingdom) (Anonymous, 1999a), but in Scotland in the same year, that figure was 9.85 per 100,000. In the United States, the incidence of cases of *E. coli* O157:H7 was 2.8 per 100,000 in 1998. Much more recent information

from Denmark indicates an incidence of 1.0 per 100,000 in 1999, and for England and Wales in the same year the incidence was approximately 2.0 per 100,000.

As methods of detecting these organisms improve and more detailed examination and investigation of cases of bloody diarrhoea occur, the reported incidence may increase.

From 1982 to 1997, more than 100 outbreaks of infection from *E. coli* O157 were reported in the United States. Where the vehicle of infection was identified, 52% were foods derived from cattle, 16% person-to-person spread, 14% fruit and vegetable produce, 12% water and 5% other foods (World Health Organization, 1997).

Figure 1
Simplified diagram of the current main routes of transmission of EHEC



ILLNESS CAUSED BY EHEC

Some people may carry EHEC as part of their transient gut microflora. When these organisms do cause illness, very serious illness can occur including haemorrhagic colitis and haemolytic uraemic syndrome (HUS). This latter condition usually occurs in children under 5 years of age and is the major cause of acute renal failure in children in Britain and several other countries. About 5% of cases of haemorrhagic colitis progress to HUS in which the case fatality rate is approximately 10% (Anonymous, 1995). Long-term sequelae in survivors of HUS may involve kidney disease, hypertension and nervous system disorders.

Some outbreaks of food poisoning caused by EHEC have involved very large numbers of people and have had particularly devastating consequences to some of those involved. In an outbreak attributed to radish sprouts in Japan in 1996, more than 6,300 school children were affected with three deaths (Fukushima *et al.*, 1997). In the United States in 1999, more than 1000 people (11 children with HUS and two deaths recorded) were believed to have been infected by contaminated water used for ice, snow cones, and lemonade (Charatan, 1999).

Although *E. coli* O157:H7 has been accorded the most attention by researchers in the clinical and food-related public health sectors, a large number of other VTEC serogroups have also been associated with foodborne illness, e.g. O26:H11, O103:H2, O111:H2, and O145:H28.

Caprioli *et al.* (1997) noted that some 10–30% of HUS in Germany, Italy, and the United Kingdom resulted from non-O157 VTEC infection and that HUS has been caused by *E. coli* O111:H– in Italy and France. Also, sudden increases in infections caused by *E. coli* O103 and O26 were noted in Germany and Italy in 1996. Beutin *et al.* (1998) reported 15 different typable non-O157 O-groups from 89 VTEC-infected patients in 1996. Thus, any strategies implemented to control *E. coli* O157 in food production and processing systems must also control all of these other, similarly dangerous organisms.

FOODBORNE OUTBREAKS AND PRODUCTS AT RISK

Table 2 indicates a variety of food types that have been linked to EHEC outbreaks, to which can be added cantaloupe melon, salad dressing containing mayonnaise made in store and cooked ham. Some possible underlying reasons for the outbreaks are also given in the table. It is striking that all of the outbreaks are probably linked to contaminated primary raw food material, whether it was meat, milk, fruit or vegetables. Adequate hygienic measures applied at the primary source and during further handling/processing, together with well-controlled and appropriate heat processes (where these were applicable), could well have, or are even likely to have, prevented the outbreaks.

It is clear that raw foods such as meat, milk, vegetables, salad, sprouted vegetables, fruits and foods exposed directly or indirectly to animal faecal contamination, e.g. washed in water contaminated by animal faecal matter, are all potential hazards for EHEC. When carrying out a hazard analysis for food production safety purposes, it is advisable to assume that all raw food may be contaminated by these organisms from time to time. Fermented food products made from raw milk or raw meat that receive no bacterial reduction process, e.g. a pasteurisation step, during manufacture will also present potential EHEC hazards.

Because the infective dose of EHEC is low, i.e. <100 cells of the organism (Advisory Committee on the Microbiological Safety of Food, 1995; Bolton *et al.*, 1996), even low levels of contamination in raw foods are of concern. Therefore, any EHEC control measures implemented in the food chain should aim not only to reduce the hazard, but to actually remove it.

Table 2
Examples of outbreaks of foodborne illness caused by VTEC and possible reasons for their occurrence (adapted from Bell and Kyriakides, 1998)

Outbreak	Organism	Products	Technology Sector	Possible Reasons for Occurrence
1992–1993 United States	*E. coli* O157:H7, STX1+, and STX2+ (VT1+, VT2+)	Cooked beefburger patties from a restaurant	Raw, comminuted meat production	Use of contaminated beefburger patties Inadequate cooking of beefburgers in the restaurant Survival of *E. coli* O157 in cooked ready-to-eat burgers
1994–1995 Australia	VTEC O111:NM	Raw fermented meat product	Fermented meat processes	Contamination of raw meat mix Inadequate process control Cross-contamination to finished product
1991 and 1996 United States	*E. coli* O157:H7	Unpasteurised, unfermented, fresh-pressed apple juice	Fresh fruit juice production	Use of fallen apples Contamination of apples with cattle manure used as fertiliser or from cattle grazing in the orchards Inadequate washing of apples Survival of *E. coli* O157 in apple juice at low pH and chilled temperature
1996 United States	*E. coli* O157:H7	Mesclun lettuce	Raw salad vegetable production	Contamination of growing crop from water supplies used for adjacent cattle ranch and lettuce growing fields Contamination of growing crop from free-range chickens that had access to cattle and lettuce fields Contamination from process wash water Inadequate staff hygiene facilities
1996 Japan	*E. coli* O157:H7, STX1+ and STX2+ (VT1+, VT2+)	School lunches containing radish sprouts	Raw salad vegetable production	Contamination of the seed Survival of seed washing Growth during seed germination

SURVIVAL, STRESS RESPONSE AND GROWTH IN FOOD

The general growth-limiting characteristics of pathogenic *E. coli* are shown in Table 3. Under freezing conditions, non-pathogenic *E. coli* are reduced 10-fold at –25.5°C over 38 weeks but little or no change in population numbers was noted for *E. coli* O157:H7 in ground beef over 9 months at –20°C (Doyle and Schoeni, 1984). In common with other vegetative bacteria, the conditions of freezing and thawing (rates, method, temperature achieved, etc.) affect the degree of injury and death of cells of *E. coli*, but it is clear that survival of freezing processes can and does occur.

Table 3
Growth-limiting parameters for pathogenic E. coli
(adapted from International Commission on Microbiological Specifications for Foods, 1996)

	Minimum	Maximum
Temperature (°C)	c. 7-8[a]	c. 44-46[b]
pH	4.4[c]	9.0
A_w	0.95	-
Sodium chloride (Glass *et al.*, 1992)	Grows vigorously in 2.5% NaCl Grows slowly in 6.5% NaCl Does not grow in 8.5% NaCl	

a growth has been demonstrated in milk at 6.5°C (Kauppi *et al.*, 1996).
b for some strains of EHEC, the maximum temperature for growth is lower than 44°C.
c *E. coli* O157 is reported to survive at pH levels below 4.4 (Semanchek and Golden, 1996).

Previous research using an increase in numbers of colony-forming units as a measure of growth demonstrated that *E. coli* do not grow under the refrigeration temperature conditions commonly used in the food industry, i.e. 2–5°C. Recent work demonstrated, however, that *E. coli*, including isolates of *E. coli* O157, are able to form long filaments under such conditions and in other conditions just outside the growth range of these organisms (T.J. Humphrey, unpublished data, 2000). The filaments seem to be similar to those formed by *Salmonella* spp. (Phillips *et al.*, 1998) including *S.* Typhimurium DT104. The public health implications of these observations have yet to be elucidated. Pathogenic *E. coli* can survive well for several weeks at refrigeration temperatures with only a 0.5–1.5-log cycle reduction in populations over 1–5 weeks (International Commission on Microbiological Specifications for Foods, 1996). *E. coli* O157:H7 survived largely unchanged in numbers in raw cow's milk held at 7°C for 144 hours and to increase 2.0 $\log_{10}$ at 15°C after 144 hours (Heuvelink *et al.*, 1998).

Pathogenic *E. coli* are not particularly heat resistant and in common with other Gram-negative pathogens, thermal inactivation / heat resistance is affected by other prevailing factors such as lowering pH (increases sensitivity to heat), water activity (a_w) and humectant used, high fat content (can increase heat resistance), and pre-conditioning of the organism, e.g. previous exposure to stress conditions (prior exposure to mild heat, i.e. heat shock). Outbreaks linked to under-cooked burgers led to research on the heat resistance of the organism in meat products. *D* values <1 minute at 63°C were generally found for *E. coli* O157:H7 in ground beef with fat contents up to 20%.

For acidic products such as cheese or fermented meats in which lactic acid is produced by the metabolic activity of the starter cultures used, or salad dressings in which acetic acid is added directly to an oil component, pH contributes to controlling EHEC growth. Survival, however, depends on the type of acid present, e.g. acetic, lactic, citric, propionic, and other physico-chemical conditions. Outbreaks in the United States linked to unfermented apple juice products known as apple cider (in Europe, cider is a fermented apple, alcoholic drink) and mayonnaise led to studies of the survival of VTEC in acidic foods. In general, at low temperatures (7–8°C), *E. coli* O157:H7 survived in such environments at pH ~4.0 for 20 days or more (Table 4).

Table 4
The survival of E. coli *O157 in acidic products*

Suspect Food	**pH and Other Conditions**	**Experimental conditions**	**Survival**	**Reference**
Fermented dry sausage	Final pH 4.4, titratable acidity 1.4%	Sausage made without starter culture, fermented, dried, and stored at 4°C for 2 months	*E. coli* O157 population reduced from 4.68 $\log_{10}$ cfu / g to 2.72 $\log_{10}$ cfu / g after 8 weeks	Glass *et al.*, 1992
Unpasteur-ised apple cider	3.6–4.0	In ciders with no preservative added Product held at 8°C after initial inoculation with ~10^5 cfu / ml. Product held at 25°C,	 *E. coli* O157:H7 could be detected for up to 20 days Survivors were detected at 2 and 3 days, but not at 6 days after inoculation.	Besser *et al.*, 1993 Zhao *et al.*, 1993
Live yoghurt locally produced from pasteurised full fat milk	The final product pH was 4.5 in the traditional and 4.6 in the "bifido" yoghurt.	*E. coli* O157:H7 (low inoculum [10^3 cfu / ml] and high inoculum [10^7 cfu / ml]) was added at the same time as the starter cultures for making traditional and "bifido" yoghurts	At both inoculum levels, populations reduced to ~10% of the initial level after 7 days stored at 4°C	Morgan *et al.*, 1993 Massa *et al.*, 1997
Mayonnaise and mayonnaise-based dressings and sauces	3.65–4.44	Three different strains of *E. coli* O157:H7 inoculated into mayonnaise at levels of ~10^7 cfu / g	Rapidly died off when stored at 25°C but cells were still detectable in mayonnaise up to 35 days when held at 7°C	Weagent *et al.*, 1994

Note: Compared with Salmonella, *survival of* E. coli *O157 in acidic conditions is greater.*

Likewise, outbreaks linked to fermented meat products, e.g. salami, prompted studies on survival during the manufacturing process and throughout the life of these products. In the United States research was carried out to determine which fermented meat processes would achieve a 5-log reduction in contaminating *E. coli* O157. For processes without a heating stage, a pH of 4.6 after fermentation was found to be required together with holding at 32.2°C for 6 or more days or 43.3°C for 4 or more days for products in small or large casings respectively (Anonymous, 1996).

More recent investigations indicate that when subjected to sub-lethal stress conditions, e.g. pH 4.0, strains of *E. coli* O157:H7 exhibited significantly greater resistance to low pH, high salt (20% w/v) and heat (56°C for 80 minutes) than did controls maintained at pH 7.0 (Rowe and Kirk, 1999). This type of cross-protection created as a response to stress is of concern in food-processing environments, where any localised contamination with these organisms may be exposed to sub-lethal concentrations of the inhibitory substances used in a food formulation and may subsequently cross-contaminate foods in production. This potential must be recognised and combated via hygienic procedures put in place to reliably eliminate foci of infection in the production environment and production equipment.

The water activity of a food product is lowered by the addition of sodium chloride, sugars and/or other solutes. The higher the concentration of the solute, the lower the water activity of the product. For products in which the water activity approaches or is lower than the minimum for growth of EHEC, growth of the organism is prevented or minimised. Hinkens *et al.* (1996) reported that *E. coli* O157:H7 survived the fermentation and drying processes of a dry fermented meat product that reached a water activity of 0.87 and pH 4.74 at the end of the process. Such reports and recent outbreaks of infection with *E. coli* O157:H7 associated with dry fermented meat products of relatively low water activity (approximately 0.9) serve to underline the fact that this organism survives the fermentation and drying processes used to produce some of these products.

In other studies, *E. coli* O157:H7 was found to survive in river water, soil cores and cattle faeces for times ranging from 2 weeks to 2 months; to grow in sprouting seeds and on prepared pre-packed fresh vegetables; to survive the cheddar cheese ripening processes up to 60 days; and to survive raw meat (salami) fermentation processes and in dried foods. Dried foods, especially those used as raw materials/ingredients in others foods, are of particular concern because if they are contaminated with EHEC and added to foods that have water activities in the range of growth of the organisms, a significantly greater hazard can result.

Studies on bacterial penetration of and/or attachment to meat surfaces and means for reducing levels of bacteria including *E. coli* O157:H7 in beef tissue indicate that bacteria can penetrate deeper layers of muscle tissue and that such penetration and attachment, particularly to collagen fibres, appears to reduce the effectiveness of both carcass and meat rinsing treatments (Fratamico *et al.*, 1996, Woody *et al.*, 2000), thus facilitating the survival of EHEC. In addition to the possible need for research directed towards seeking antimicrobial rinses effective against bacteria that are attached to meat surfaces, practices at the primary stages of the slaughter and carcass dressing process should clearly continue to be a target for improvement. These are important critical points at which much can be done to minimise the potential for bacteria to contaminate, attach to, and penetrate carcass meats in the first place.

Clearly, difficulties in removing EHEC once present on meat and the tolerance of EHEC to a wide range of pH and water activity conditions and low temperatures, including freezing, mean that there is considerable potential for these organisms to survive in many food environments and remain present in or on many food types. Because only low numbers of viable cells are necessary to cause illness, knowledge and application of relevant controls at all stages of food production are important if food safety is to be improved in relation to this hazard.

LABORATORY METHODS

Conventional methods for the routine isolation and identification of normal *E. coli* are ineffective for the isolation of *E. coli* O157 for which specific methods of isolation have been developed. The most successful and widely used of these methods employs selective culture enrichment of the organisms in the food sample, followed by capture and concentration of cells of *E. coli* O157 from the enrichment broth by using immunomagnetic particles coated with a specific antibody (immunomagnetic separation, or IMS). The captured cells on the particles are transferred and spread onto the surface of selective agars and incubated. Colonies growing on the agars showing typical characteristics of *E. coli* O157 are further tested to confirm their identity (International Standard, ISO 16654 Draft, 1999).

Although in some countries, e.g. the United States and the United Kingdom, more than 90% of illnesses caused by EHEC are due to *E. coli* O157:H7, in other countries, non-O157 EHEC are important causes of outbreaks. It is now recognised that it is important to have methods in place for specifically isolating and identifying non-O157 EHEC. Compared with method development for the detection and characterization of *E. coli* O157, relatively little has been done so far to develop routine methods for detecting non-O157 EHEC. Consequently, more research is required to develop suitable methods for the specific detection and characterisation of these organisms.

CONTROL

Because of the widespread environmental sources and survival characteristics of EHEC, their presence at low frequencies and levels in raw foods, particularly of red meat and vegetable origin, must be expected.

It is essential that responsibility is exercised at each step of the food chain to ensure that relevant controls are in place either to prevent / minimise contamination of food materials by EHEC or to prevent the further growth, or preferably reduce the numbers, of EHEC already present.

Governments and public health enforcement agencies alone are not in a position to ensure the production of safe foods. Individuals directly involved in the primary production, processing, retailing, catering, and domestic handling of food hold the keys to safe food production. The role of governments and their agencies should be to facilitate the provision and dissemination of accurate and relevant microbiological information that can be used in risk assessments by food industry personnel and to ensure that legislation is even-handed (applied to both large and small food businesses), is soundly based on HACCP systems, and includes an obligation for all food businesses to demonstrate appropriate knowledge, competence, facility, etc., before being allowed to commence business.

Guidelines for the control of infection with EHEC such as those produced recently by a sub-committee of the Public Health Advisory Committee on Gastrointestinal Infections (Anonymous, 2000) and the Food Safety Authority of Ireland (Anonymous, 1999 a–g) are to be welcomed and endorsed. However, it is also vitally important to strengthen and maintain, on a global basis, the co-operation necessary among food producers, processors, retailers, caterers, governments and consumers to effect practical controls that will successfully minimise (if not eliminate) the hazard from these organisms.

The general approach taken in European and North American legislation clearly places the responsibility on food business proprietors to produce and supply safe and wholesome foods and to employ HACCP-based systems to do so, e.g. European Union Directive 93 / 43 / EEC, 14th June 1993, on the hygiene of foodstuffs, Article 3, paragraph 2 (Anonymous, 1993). Simple failures of basic food safety requirements, however, have resulted in outbreaks of illness from EHEC (Table 2). Such failures were avoidable and should have been prevented.

In the food production chain, a number of controls are globally applicable to the reduction / elimination of EHEC (see Annex 1, General Principles for Safe Food Production).

CONCLUSION AND RECOMMENDATIONS

Although a great deal of work has been carried out on all aspects of *E. coli* since it was first described, the organism continues to provide new challenges largely because of the constant evolution of types within the species and the development of types that cause illness from extremely low infective doses, e.g. <100 cells. EHEC are the latest and most serious of these new challenges, and illness caused by EHEC is largely preventable by good hygienic practice and thorough cooking processes.

Far more work must be done to increase our knowledge and identify the reservoirs and routes of transmission to humans of the different EHEC serotypes. However, it will always be prudent to assume that these organisms are present in raw foods at some low frequency and level. Thus, the introduction and consistent maintenance of basic practical hygiene and process control measures by all persons directly involved at all stages of the food production chain should prevent any complacency that could lead to further rises in cases of foodborne illness caused by EHEC.

The basic requirements for keeping the food supply safe from the hazard of EHEC are known and are straightforward. It is necessary through an active partnership involving veterinarians, medical practitioners, epidemiologists, and the food industry (from producers to retailers and caterers) to ensure that these basic requirements are globally met.

International cooperation fostered by governments is essential to ensure the common understanding and application of the basic controls necessary to prevent EHEC from becoming a major scourge of this new century. The following recommendations are made:

1. Research requirement: develop simple methods for the routine detection of all EHEC types.
2. Research requirement: employ microbiological surveillance of agricultural and aquatic environments and raw foods, using the techniques developed in recommendation 1, to determine the full EHEC cycle and potential break points.
3. Research requirement: determine the foodborne hazards from non-O157 EHEC organisms and non-*E. coli* Shiga toxin-producing organisms.
4. Under the auspices of international bodies, e.g. Codex Alimentarius Commission or the World Health Organization (WHO), a select working party should be charged with the task of managing the production of:
   - internationally applicable, sector-related, e.g. raw meat, dairy products, raw fermented meat, guides to effective EHEC controls that can be practically implemented at all relevant levels of the food production chain, and
   - a relevant consumer education package.
5. Under the auspices of international bodies, e.g. Codex Alimentarius Commission or WHO, an international work programme should coordinate results from research, surveillance projects, and outbreak investigations relating to EHEC to ensure that results obtained can be readily understood and applied by other workers.
6. Governments should apply food safety legislation equally to all food businesses throughout the food chain, from primary agriculture / aquaculture to retailers and caterers regardless of the size of the business.
7. Use current and future scientific knowledge in risk assessments to ensure that all reasonable practical measures are taken to keep the food supply safe from EHEC.

GLOSSARY

Bacteriophage Viruses whose hosts are bacteria, i.e. bacterial viruses.

Biotyping The conventional method for distinguishing between bacterial types using their metabolic (biochemical reaction changes caused) and / or physiological properties.

Challenge test A study in which the organism of concern, e.g. *E. coli* O157:H7, is inoculated and distributed at different levels into real food products. Specific numbers of inoculated and un-inoculated (control) products are usually then incubated at different temperatures and the levels of the organism are determined at different time intervals. Alternatively, the organism may be inoculated into a raw material mix, e.g. for salami products, and the product is then processed through the required manufacturing conditions. Again, the levels of the organism are determined at intervals throughout the process and storage time. Results are commonly used to determine process or shelf life conditions that will deliver a safe product with respect to the bacterial hazard studied, i.e. VTEC. These studies should be carried out only in research laboratory facilities and by people qualified and experienced in designing studies relevant to the target product types and organisms.

Critical control point (CCP) A step at which control can be used to prevent or eliminate a food safety hazard or reduce it to an acceptable level.

***D* value** The time required (usually expressed in minutes) at a given temperature to reduce the number of viable cells or spores of a given microorganism to 10% of the initial population.

Facultative Optional lifestyle, associated with the mode not normally adopted, e.g. facultative anaerobe is a microorganism that is usually aerobic but can grow anaerobically.

Genotyping Methods used to differentiate bacteria based on the composition of their nucleic acids.

HACCP Hazard analysis critical control point: system which identifies, evaluates and controls hazards which are significant for food safety.

Hazard A biological, chemical or physical agent in, or condition of, food with the potential to cause an adverse health effect (Codex Alimentarius Commission, 1999).

Hazard analysis The process of collecting and evaluating information on hazards and conditions leading to their presence to decide which are significant for food safety.

Infection In relation to bacterial food poisoning, a condition in which a pathogen multiplies in the host's body and becomes established in or on the cells or tissues of the host.

Pasteurisation A form of heat treatment that kills vegetative pathogens and spoilage microorganisms in milk and other foods, e.g. for milk, the legal requirement for the pasteurisation process in the European Union is at least 71.7°C for 15 seconds.

Pathogen Any microorganism which causes disease in humans or animals by direct interaction with (infection of) the host.

Pathogenic Pertaining to behaviour as a pathogen.

Phage typing A method used to distinguish among bacteria within the same species on the basis of their susceptibility to a range of bacterial viruses (bacteriophage).

Phenotype The observable characteristics of an organism, including biotype, serotype, phage type, and bacteriocin type.

Plasmid Extra-chromosomal DNA that replicates independently of the host cell DNA. Plasmids (and the genetic characteristics they carry) are transferable between cells.

Polymerase chain reaction (PCR) A technique used to amplify the number of copies of a pre-selected region of DNA to a level sufficient for testing.

Polymorphism The ability to occur in two or more morphologically distinct types (morphotypes), depending on prevailing conditions.

Pulsed field gel electrophoresis (PFGE) A technique which allows chromosomal restriction fragment patterns to be produced from restriction enzyme analysis.

Restriction enzyme analysis (REA) A method for discriminating among isolates of the same species on the basis of patterns obtained from the separation of DNA fragments in agarose gel after they have been digested with one or more restriction enzymes, e.g. EcoR1, HaeIII, HhaI, or Xhol. Differences in the banding profiles of two isolates are referred to as a restriction fragment length polymorphism.

Risk The probability of an adverse health effect and the severity of that effect consequential to a hazard(s) in food.

Serotyping A method of distinguishing among bacteria on the basis of their antigenic properties (reaction to known antisera). The O antigen defines the serogroup of a strain and the H antigen defines the serotype of the strain; a number of serotypes may thus constitute a serogroup.

STEC Shiga-like toxin-producing *E. coli.*

Strain An isolate or group of isolates that can be distinguished from other isolates of the same genus and species by either phenotypic and / or genotypic characteristics.

Thrombocytopaenia Low numbers of platelets circulating in the blood stream.

Verocytotoxigenic Pertaining to organisms which produce a toxin capable of killing vero cells, an established cell line derived from African Green Monkey kidney.

Virulence The capacity of a pathogen to cause disease, generally expressed in relation to the severity of the illness caused in the host, e.g. a highly virulent strain may cause very severe illness.

VTEC Verocytotoxin-producing *E. coli*, i.e. *E. coli* that produce verocytotoxin (Shiga toxin) that destroys vero cells.

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ANNEX 1

General Principles for Safe Food Production[1]

General Principles

Environment

- Ensure that human and animal waste and slurries are effectively treated to eliminate, or at least minimise, the bacterial pathogen load prior to discharge into the environment and surface waters.

Manufacturing site

- Ensure that all food production, processing and handling facilities (building environment, equipment, etc.) are suitable for the purpose required.
- Ensure that adequate hygienic facilities are provided for staff.
- Ensure that sufficient cleaning facilities are provided to allow maintenance of high standards of environment, equipment, and personal hygiene.
- Ensure that potable water supplies are available and used for all food contact purposes.

Staff

- Ensure that an employee health programme is in place for food handlers.
- Ensure that suitably qualified staff are employed for the tasks required.
- Ensure that all staff are properly trained in all aspects of personal hygiene, particularly effective hand-washing procedures.
- Ensure that all staff are properly trained for the job they are required to do, particularly in aspects affecting process critical control points.
- Ensure that all staff are provided with the necessary tools to carry out the tasks expected of them.

Quality systems

- Ensure that appropriate food safety management tools, e.g. HACCP-based approaches, are employed by suitably trained and qualified people to establish relevant EHEC control points, and maintain appropriate means for monitoring these.
- Operate an appropriate quality assurance programme for raw material suppliers.

Processes

- Ensure that, where applicable, temperatures (chill or heat processing) are consistently and accurately maintained.

New product and process development

- Ensure that before the application of any new technology, alternative technology or the new application of an existing technology (whether in food processing, food packaging, storage, or any combination of these), any consequent effect on EHEC reduction, destruction, survival, etc., is properly considered and evaluated in a structured hazard analysis.

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1. *General reference: Codex Alimentarius Commission (1997) International Code of Practice – General Principles of Food Hygiene, CAC/RCP 1-1969, Rev. 3, 1997, Codex Alimentarius Commission, Rome.*

- For products and processes that may be considered marginal in relation to the destruction of EHEC or where survival may be possible, it may be appropriate to perform challenge studies specifically designed to determine the process effects on the survival of EHEC to demonstrate that the final product will be safe with respect to EHEC hazards.

Specific matters

Primary production

- Avoid the use of untreated animal or human faeces / manure as fertilisers.
- Avoid the use of faecally contaminated water supplies to irrigate fruit and vegetable crops.
- Provide adequate staff facilities for hand-washing during harvesting of crops that are destined to be eaten without further processing (other than washing), with any crop washing carried out on the farm done using potable water supplies.
- Ensure that all milking animals are adequately cleaned before milking and that all milking equipment is kept, used and maintained in a hygienic condition.
- Ensure that animals sent for slaughter are in a clean condition (as far as is practicable).

Processing

Meat

Transport

- Ensure that live animal and carcass meat transport vehicles are kept, used, and maintained in a hygienic condition.

Slaughter and raw meat butchery

- Employ pre-slaughter feeding regimes that exploit ruminant digestive systems to reduce carriage of EHEC.
- Minimise faecal contamination on hides.
- Avoid gut content spillage during evisceration.
- Employ carcass decontamination systems.
- Ensure that clean carcasses are chilled rapidly.
- Ensure that all knives, scrapers, etc., are kept in good hygienic condition.
- Employ monitoring systems / schemes aimed at detecting and controlling contamination sources.

Product manufacture

- Ensure that process flows are designed to eliminate any possibility of cross-contamination between raw and heat-processed meat / meat products.
- Ensure that process conditions are consistently maintained to achieve the physico-chemical conditions required to control (reduce / eliminate) EHEC, e.g. temperature and humidity cycles in raw, fermented meat manufacture to achieve effective pH reduction and acidity development and reduction in water activity (loss of moisture). Consider the use of challenge studies to demonstrate the effective control of EHEC, e.g. minimum 3-log reduction in the inoculated level of EHEC.

Dairy

- Ensure that where pasteurisation is used as a critical control point, systems, e.g. diversion valves, are in place and operating correctly to prevent any un-pasteurised milk going through.
- Ensure process flows are designed to eliminate any possibility of cross-contamination between raw and heat-processed milk / milk products.

- Ensure that process conditions are consistently maintained to achieve the physico-chemical conditions required to control (reduce/eliminate) EHEC, e.g. starter culture activity and incubation temperature in cheese and yoghurt manufacture to achieve effective pH reduction and acidity development. Consider the use of challenge studies to demonstrate the effective control of EHEC, e.g. a minimum 3-log reduction in the inoculated level of EHEC.

Vegetables and salad vegetables

- Washing processes should be designed to ensure that contaminated water is not re-used without treatment, e.g. removal of soil and debris, chemical (hypochlorite) dosing.
- Ensure that process flows are designed to eliminate any possibility of cross-contamination between raw and heat-processed vegetables/vegetable products.
- Ensure that water used for "refreshing" heat-processed vegetables is of potable quality.
- Ensure that process conditions are consistently maintained to achieve the physico-chemical conditions required to control (reduce/eliminate) EHEC e.g. pH, temperature, and equilibration of acidity in acidified vegetable and vegetable/mayonnaise product manufacture. Consider the use of challenge studies to demonstrate the effective control of EHEC, e.g. minimum of 3-log reduction in the inoculated level of EHEC.

Fruit-based products

- Avoid the use of "dropped" fruit and obviously faecally contaminated fruit for products destined to be consumed as "natural" products, e.g. un-pasteurised fruit juices or raw as a component of products.
- Ensure that process flows are designed to eliminate any possibility of cross-contamination between raw and heat-processed fruit products.
- Pasteurise products where possible.

Retailing

Food counters, butcher shops, delicatessen counters, market stalls

- Ensure that raw and cooked foods, whether in refrigerated storage or on counters, are completely separated.
- Ensure that adequate food-handling equipment, including scales, is provided to allow raw and cooked foods to be handled completely separately.
- Ensure that customers receive adequate instructions for the safe handling and storage of all food types and, where applicable, cooking instructions, especially for raw meats and raw meat products.

Catering

- Ensure that raw and cooked foods, whether in refrigerated storage or on counters, are completely separated.
- Ensure that adequate food-handling equipment is provided to allow raw and cooked foods to be handled completely separately.
- Ensure that all cooking procedures for raw meats and vegetables are adequate for the complete destruction of *E. coli*, e.g. to achieve at least a 10^6-fold (6 *D* value) reduction of EHEC in beefburgers, a heat process equivalent to 70°C (internal temperature) for 2 minutes is generally advocated.
- Ensure that post-cooking procedures will not allow re-contamination.

In the home

Ensure that foods are always handled safely, i.e.:

- Hands are always washed properly prior to preparing food and between preparations of different foods.
- All food is stored in clean conditions.
- Raw food is stored separately from cooked / ready-to-eat foods.
- Refrigerators are packed correctly, e.g. raw foods should be stored below cooked / ready-to-eat foods.
- Separate chopping / cutting boards are used for raw and cooked / ready-to-eat foods.
- All food surfaces are kept clean and sanitised.
- Cooking methods achieve thorough cooking of meat and vegetables.
- Leftover cooked food is cooled quickly, refrigerated, and consumed as quickly as possible.

Governments

- Consistent and correct food safety information needs to be available to food handlers and the public. Governments should ensure that correct key food safety information is provided and that it is used consistently by manufacturers, retailers, professional bodies, training organisations, etc., to inform staff and consumers.
- Governments should work in partnership with industry to coordinate the production of food sector-related international guidelines and codes of practice aimed at eliminating food-related EHEC illness.

ANNEX 2

Fact Sheet on Entero-haemorrhagic Escherichia coli (EHEC)

Summary

Strains of entero-haemorrhagic *Escherichia coli* (EHEC) are a major global food safety concern because they are widespread in the environment via their natural habitat in cattle and other animals and because severe illness can result from the ingestion of very few cells. The importance of EHECs other than *E. coli* O157:H7 has yet to be determined, but current well-managed food production processes, e.g. cleaning procedures, pasteurisation heat processes, can reduce these hazards in many foods. Potential EHEC hazards can be expected in raw foods such as meat, milk, vegetables, salad, sprouted vegetables and in fruits, and foods exposed directly or indirectly to animal faecal contamination, and also in fermented food products made from raw milk or raw meat that receive no bacterial reduction process, e.g. a pasteurisation step during manufacture. An active partnership among governments, food producers and processors, and consumers needs to be developed and maintained to ensure the reliable control of EHEC throughout the food production chain.

The organism

EHEC are a highly virulent, pathogenic sub-group of *E. coli*. Many *E. coli* are commonly harmless bacterial members of the normal microflora of the intestinal tract of humans and other warm-blooded animals. Only very few EHEC cells, i.e. less than 100, are able to cause illness in humans.

Spread of EHEC in the food chain

The EHEC "cycle" in the environment is probably driven primarily by bovine faecal contamination, which contributes to widespread faecal contamination of the agricultural environment and, hence, can affect a wide variety of raw foods. In addition, many wild animals and birds are also now known to be carriers of verotoxin-producing *E. coli* (VTEC) pathogenic to humans, and they perpetuate the reservoir of these organisms in the environment and add to the sources of contamination of the raw food supply.

Foods at risk

Foods exposed directly or indirectly to animal faecal contamination, e.g. foods that are washed in water contaminated by animal faecal matter, present potential hazards. Raw meats (particularly beef), raw milk and milk products, and raw produce and fruit all may, from time to time, be contaminated with EHEC. Other foods that present potential EHEC hazards include fermented food products made from raw milk such as raw milk soft cheeses, and raw meat such as salami products that receive no bacterial reduction process, e.g. a pasteurisation step, during processing. When carrying out a hazard analysis for food microbiological safety purposes, it is advisable to assume that all raw food may be contaminated by these organisms from time to time.

Severity of illness and treatment

EHEC cause some very serious illnesses, including haemorrhagic colitis and haemolytic uraemic syndrome (HUS). This latter condition usually occurs in children under 5 years of age and is the major cause of acute renal failure in children in Britain and several other developed countries. Up to 10% of patients infected with VTEC O157 may develop HUS. Infection with VTEC O157 can result in death, and although rates of fatality vary considerably, they can be high, e.g. above 9%, particularly where institutional outbreaks occur. Generally, about 5% of cases of haemorrhagic colitis caused by VTEC progress to HUS, in which the case fatality rate is approximately 10%. Long-term illness in survivors of HUS may involve kidney disease, hypertension, and nervous system disorders.

The diarrhoeal phase of the infection caused by EHEC is usually self-limiting and there are no specific treatments of the conditions caused by the organism. Each symptom is treated as it occurs in an individual. The usefulness of antibiotics in controlling the course of the illness is controversial and is usually contra-indicated because such treatment is believed to increase the risk of the patient developing HUS (Wong *et al.*, 2000). In addition, there is growing evidence of an increasing prevalence of antimicrobial-resistant strains of VTEC O157. Such information is of concern to the food industry, which is already having to deal with the implications of multiple antibiotic-resistant strains of *Salmonella typhimurium* DT104.

Incidence in man

Compared with *Salmonella* and *Campylobacter*, the reported incidence of EHEC is generally low, e.g. approximately 2 per 100,000 in England and Wales in 1999, 1 per 100,000 in Denmark in 1999, 2.8 per 100,000 in the United States in 1998, and 4.1 per 100,000 in Canada in 1996. In most developed countries for which data are available, the incidence is generally higher now (four times higher in the United Kingdom) than it was in 1990, and will probably continue to increase. The severity of the illness and the high fatality rate make the organism significant in public health and food safety terms.

Survival and growth of EHEC in food

Information from outbreaks and research indicates that some strains of EHEC survive well in some foods at low pH and/or in low water activity environments, e.g. raw fermented meats and apple cider. However, they are not particularly heat resistant and properly controlled pasteurisation processes kill these organisms.

Because EHECs are tolerant to a wide range of pH and water activity conditions and low temperatures, including freezing, there is considerable potential for these organisms to survive in many food environments and remain present in or on many types of foods.

Control of EHEC in the food chain

Because of the widespread environmental sources and survival characteristics of VTEC, their presence at low frequency and levels in raw foods, particularly red meats and vegetables, must be expected. It is essential that at each step of the food chain responsibility be exercised by all those involved, including producers, processors, transporters, retailers, caterers and consumers, to ensure that relevant controls are in place that will either prevent or minimise contamination of the food materials by EHEC and that any present will, at least, be prevented from further growth or, preferably, be reduced in numbers. An active partnership among governments, food producers and processors and consumers needs to be developed and maintained to ensure that correct information is available and disseminated to promote the reliable control of EHEC throughout the food chain.

Laboratory isolation

Conventional methods to detect and enumerate "normal" *E. coli* do not detect EHEC. Thus, special selective methods must be used and only laboratories with high levels of protection must be used to handle isolated EHEC. Full characterisation of these organisms is best carried out by specialists in public health laboratories.

Outbreaks

Foods implicated in EHEC outbreaks have been raw foods / drinks contaminated at source, e.g. apple juice and sprouting vegetables, foods produced in processes where failures have occurred, e.g. pasteurisation, and contaminated raw foods (to be eaten after cooking) that were improperly cooked prior to consumption, e.g. beefburgers.

Conclusions and implications for the future

Entero-haemorrhagic *E. coli* are present at low levels and frequencies in many raw foods. Many of the measures applied in the food industry to control other enteric pathogens, e.g. *Salmonella*, also control EHEC. Nevertheless, much greater effort is required in the agricultural sector to minimise the levels of EHEC gaining entry into the food chain in the first place, especially because only very low levels of these organisms are required to cause illness. In addition, more knowledge is required about the public health implications of EHEC other than *E. coli* O157:H7, and a more concerted effort must be made by governments, industry and consumer groups to ensure that at all levels of food production, handling, and consumption, relevant information is available to enable everyone to practice safe food handling and personal hygiene to control this most serious of bacteriological hazards.

ANNEX 3

Questions and Answers

Entero-haemorrhagic Escherichia coli (EHEC)

What are EHEC?

Entero-haemorrhagic *Escherichia coli* (EHEC) are a pathogenic and highly virulent subgroup of *E. coli*. Other subgroups of *E. coli* bacteria range from harmless members of the normal gut flora of humans and animals to those that can cause illness in humans and animals.

Why are they important?

EHEC are important because they

- are highly infectious in humans,
- have a very low infectious dose,
- cause serious acute illness,
- cause serious long-term sequelae, especially kidney failure,
- are naturally occurring in cattle (and other animals) and, hence, the soil,
- are global.

Where do they come from?

The primary habitat of EHEC is the intestinal tract of cattle and other warm-blooded animals. EHEC infections in humans are transmitted directly from animals, from person to person, and via contaminated foods. Widespread faecal contamination of the environment (soil and water) by farm and wild animals provides a continuing source of EHEC in the agricultural environment and, hence, to a wide variety of raw foods. Raw meats (particularly beef), raw milk and raw milk products, and raw produce and fruit may all be contaminated with EHEC from time to time.

What can be done about EHEC?

Cattle and dairy farms

Control slurry disposal and prevent contamination of waterways. Use techniques for reducing pathogen loads in animal wastes, e.g. composting and anaerobic digestion. Prevent contamination of feed. Keep animals clean prior to and during milking and when delivered for slaughter. Employ pre-slaughter feeding regimes that exploit ruminant digestive systems to reduce EHEC carriage.

Slaughterhouse

Ensure that animals are as clean as possible, i.e. minimise obvious faecal soiling, prior to slaughter. Use clean equipment and practices that prevent contamination between carcasses. Minimise any aerosol dispersion from the use of hoses.

Meat-cutting plant

Keep workstations and equipment clean and use practices that minimise contamination between meat cuts and animals species.

Vegetable growing and processing

Do not use untreated animal or human wastes as fertilizer for growing salad crops to be eaten raw. Do not allow grazing animals access to fields where vegetables are grown for human consumption. Minimise soil brought into the processing plant on the vegetables. Use chlorinated water for all in-plant vegetable washing processes, being sure to maintain a residual level of available chlorine. Avoid the build-up of debris in or on all equipment and other components of the processing environment.

Fruit growing and processing

Do not use untreated animal or human wastes to fertilise orchards. Do not allow grazing animals access to orchards. Do not use dropped fruit, and exclude the use of obviously soiled fruit, particularly for products that will have no positive destructive stage during processing. Where possible, use pasteurisation to destroy EHEC. Use chlorinated water for all in-plant fruit-washing processes, being sure to maintain a residual level of available chlorine. Avoid the build-up of debris in or on all equipment and other components of the processing environment.

Further processors

Operate a quality assurance programme for raw material suppliers, including regular audits of supplying units and relevant microbiological specifications. Carry out full, formal and structured hazard analyses of all production processes for all products, and implement necessary controls and monitors of critical points determined for the control of EHEC.

Retailers

Operate a quality assurance programme for product suppliers, including regular audits of supplying units and relevant microbiological specifications. Require from suppliers full, formal and structured hazard analyses of all production processes for all products, and implement necessary controls and monitors of critical points determined for the control of EHEC. Review these analyses as part of the quality assurance programme audit. Ensure that employees are properly trained in the handling and treatment of all products and in personal hygiene standards and environmental and equipment hygiene procedures.

Provide customers with full and easily comprehended information about the safe storage, handling, and treatment of products.

Caterers

Operate a quality assurance programme for product suppliers, including regular audits of supplying units. Require from suppliers full, formal and structured hazard analyses of all production processes for all products and implement necessary controls and monitors of critical points determined for the control of EHEC. Review these analyses as part of the quality assurance programme audit. Ensure that employees are properly trained in the handling and treatment of all products and in personal hygiene standards and environmental and equipment hygiene procedures.

Consumers

Increase efforts to educate consumers about food safety:

- Always wash hands prior to preparing food and between preparations of different foods.
- Follow basic hygienic procedures for all food handling.
- Read and follow on-pack instructions and information provided by retailers.
- Keep raw foods and cooked foods well separated and use separate utensils and boards in their preparation.
- Always store foods appropriately: ready-to-eat foods should be stored at the top of the refrigerator, and raw foods at the bottom.
- Apply common sense when cleaning kitchen areas and utensils.

Governments

Governments should ensure that correct key food safety information is provided to, and then consistently used by manufacturers, retailers, professional bodies, training organisations, etc., to inform and update staff and consumers. Governments should work in partnership with industry to coordinate the production of food sector-related international guidelines and codes of practice aimed at eliminating food-related EHEC illness.

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