codex alimentarius commission

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

WORLD HEALTH ORGANIZATION

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DISCUSSION PAPER ON THE RISK PROFILE FOR ENTEROHEMORRHAGIC *ESCHERICHIA COLI* INCLUDING THE IDENTIFICATION OF THE COMMODITIES OF CONCERN, INCLUDING SPROUTS, GROUND BEED AND PORK

(Prepared by the United States, with the assistance of Australia, Austria, Canada, China, France, Germany, Japan, Netherlands and the European Union)

BACKGROUND

At its 34th session, the Committee confirmed that enterohemorrhagic *Escherichia coli* remained as a priority item of work for the Codex Committee on Food Hygiene (CCFH). The CCFH therefore agreed to have the United States prepare a risk profile with the assistance of Austria, Australia, Canada, China, France, Germany, Japan and the European Commission for enterohemorrhagic *Escherichia coli* that would include identification of the commodities of concern, including ground beef, leafy green vegetables, sprouts, and pork.

At its 35th Session, the Committee agreed to solicit comments by Circular Letter on the top five serotypes of human EHEC isolates, the top five commodities of concern and the scope of animal husbandry practices that should be included in the risk profile. The Committee also asked the United States along with its drafting partners to update the risk profile in light of comments received. This document has been revised in light of the discussion during the 35th Session, the responses to the Circular Letter and the comments received from drafting group members.

SCOPE

This Discussion Paper will provide an overview of (1) the complete risk profile which can be found in its entirety in Appendix A, (2) existing international guidance documents and codes of practice that are likely to mitigate the occurrence of human enterohemorrhagic *Escherichia coli* (EHEC) infection and (3) suggested risk management activities for consideration by CCFH. Note that this discussion paper was developed extensively from the attached risk profile.

OVERVIEW OF THE RISK PROFILE FOR ENTEROHEMORRHAGIC *ESCHERICHIA COLI* IN VARIOUS COMMODITIES OF CONCERN

1. Pathogen-food commodity combination(s) of concern

1.1 Pathogen of concern

Enterohemorrhagic *Escherichia coli* (EHEC) were first identified as human pathogens in 1982, when *E. coli* strains of a previously uncommon serotype, O157:H7, were implicated in two outbreaks of hemorrhagic colitis (bloody diarrhoea) in the United States (U.S.) (Riley et al. 1983; Wells et al. 1983). Since then, outbreaks due to this new pathogen have become a serious public health problem throughout many regions of the world (Schlundt 2001; Clarke et al. 2002). While *E. coli* O157:H7 strains are the predominant cause of EHEC infection in the U.S. and the U.K., it is important to note that EHEC strains of other serogroups, such as O26, O103, and O111, have been increasingly linked to human illness. This is illustrated by surveillance data from Japan (Table 1) and has been stated in a WHO report (WHO 1998). Furthermore, in countries such as Australia *E. coli* O157:H7 has not made a major impact, strains of serotypes O26 and O111:H- being of greater concern. However, these data notwithstanding, the preponderance of available data suggest *E. coli* strains of serotype O157 should continue to be considered the foremost concern for EHEC infection.

Most clinical laboratories do not routinely screen for non-O157 EHEC, because of the lack of a biochemical marker (Mead and Griffin 1998). While *E. coli* O157:H7 are easily differentiated biochemically from other enteric *E. coli* because they ferment sorbitol slowly, diagnostic methods for identifying non-O157 EHEC are not widely available in most laboratories; consequently infections caused by these pathogens are often not confirmed. Recently, new methods for the detection of O103, O111, O26 and O145 serogroups have been developed; these advances may facilitate the collection of more data regarding the prevalence and significance of these serotypes as it pertains to human foodborne illness (Cudjoe 2001). Mead et al. (1999) have estimated that the incidence of non-O157 EHEC is between 20% and 50% that of *E. coli* O157:H7 infection.

1.1.1 Key attributes of the pathogen including thermal stability, acid resistance and virulence characteristics.

A number of factors influence the survival and growth of EHEC in food, including temperature, pH, salt, and water activity (Meng and Doyle 1998). Studies on the thermal sensitivity of *E. coli* O157:H7 in ground beef suggest EHEC are not unusually heat-resistant and that heating ground beef sufficiently to kill typical strains of *Salmonella* spp. will also kill EHEC. The optimal temperature for growth of *E. coli* O157:H7 is approximately 37°C (98.6°F), and the organism will not grow at temperatures below 8°C to 10°C (46°F to 50°F) or above 44°C to 45°C (Doyle and Schoeni 1984; Buchanan and Doyle 1997). *E. coli* O157:H7 survives freezing, with some decline in concentration (Ansay et al. 1999). *E. coli* O157:H7 has been reported to be more acid resistant than other *E. coli*. Acid resistance enhances the survival of *E. coli* O157:H7 in mildly acidic foods and may explain its ability to survive passage through the stomach and cause infection at low doses.

Table 1: Serotypes of human EHEC isolates from 1999-2000 in Japan*

Serotype	1999 Cases (% of total)	2000 Cases (% of total)
O157	1394 (72.1)	1158 (69.9)
O26	346 (17.9)	377 (22.8)
O111	81 (4.2)	42 (2.5)
All other	112 (5.8)	79 (4.8)

*http://idsc.nih.go.jp/iasr/22/256/graph/t2563.gif

Please refer to appendix A for a discussion of virulence characteristics associated with EHEC.

1.2 Description of the food or food product and/or condition of its use with which foodborne illness due to this pathogen has been associated.

To choose the most appropriate product to consider in this risk profile, the frequency with which various products were implicated in causing EHEC infection was considered. We evaluated available studies of sporadic cases of EHEC infection and outbreak investigation reports. Food vehicles implicated most frequently were raw or undercooked foods of bovine origin, especially undercooked ground or minced beef and unpasteurised milk; however, an increasing number of outbreaks have been associated with the consumption of raw or minimally processed fruits and vegetables. Hence, due to their relevance to human cases of EHEC infection, ground beef and green leafy vegetables are the commodities of focus in this risk profile. Commodities worthy of future consideration include raw milk products, unpasteurised cider, fresh-cut fruits, and sprouted seeds.

1.2.1 Foods of bovine origin

Beef was cited as the source of 46% of foodborne outbreaks of EHEC infection with a known vehicle of transmission in the U.S. during the years 1993 to1999. Other products of bovine origin that have been implicated in a number of outbreaks of EHEC infection have included raw and improperly pasteurised cow's milk, as demonstrated by an outbreak of infection linked to *E. coli* O104:H21 in contaminated milk (Feng et al. 2001). Effective pasteurisation eliminates EHEC from milk, including *E. coli* O157:H7.

1.2.2 Foods of non-bovine origin

Fruits and vegetables contaminated with EHEC account for a growing number of recognised outbreaks (Table 2). Fresh potatoes (Morgan et al. 1988), lettuce (Ackers et al. 1998, Mermin et al. 1997, Hilborn et al. 1999), radish (Michino et al. 1999), alfalfa sprouts (Breuer et al. 2001, MMWR 1997a), and cantaloupe (Del Rosario and Beuchat 1995) have all been associated with EHEC infections. Between 1998 and 1999, leafy green vegetables were cited as the source of 26% of the foodborne outbreaks of EHEC infection with a known vehicle of transmission in the U.S. Contamination of produce may occur in several ways, including through use of manure or water contaminated with faecal matter (Solomon et al. 2002a; Wachtel et al. 2002a; Solomon et al. 2002b) and through handling by workers with poor health and hygiene. In a number of instances, manure from nearby cattle lots was suspected to be the original source of EHEC (Ackers et al. 1998; Hilborn et al. 1999). Such contamination may be spread by runoff and/or wind. When tertiary-treated sewage that had not been treated with chlorine was accidentally released, cabbage plants were found to have *E. coli* strains (not containing *stx1*, *stx2* or *eae* genes) associated with the plant roots when control fields did not (Wachtel et al. 2002a). Cross-contamination in the retail environment or consumer kitchen between contaminated meat products and produce may also occur.

Current data based on outbreaks and sporadic infections indicate consumption of ground beef remains the single most important source of foodborne EHEC infection; however, leafy green vegetables are the second most significant cause of infection, as they are vulnerable to contamination in the field or packing environment and are typically eaten raw.

Table 2: Food Vehicles Implicated in Outbreaks of *E. coli* O157:H7, U.S., 1998-2001[a]

Vehicle	1998	1999	2000	2001	Total
Ground beef/hamburger	10	9	4	4	27
Roast Beef	0	2	0	1	3
Combined green leafy vegetables	4	7	1	1	13
Salad	1	1	1	1	4
Coleslaw (cabbage)	2	1	0	0	3
Lettuce	1	3	0	0	4
Milk	2	0	0	0	2
Other	5	5	6	2	18

Total No. Outbreaks[a]	21	21	11	8	63

[a]Outbreaks for which food vehicles were known; outbreaks due to unknown vehicles were not included.

[b]Note that because 'Combined leafy green vegetables' are further grouped into three sub-categories, the numerical values for 'Total No. Outbreaks' are not the sum of the numerals in their respective columns.

Sources: CDC 1999b; CDC 2001c.

2. Description of the public health problem

2.1 Characteristics of the disease

Following ingestion of EHEC, the human response ranges from asymptomatic infection to death. The incubation period until symptom onset ranges from one to eight days. Illness typically begins with abdominal cramps and nonbloody diarrhoea that can, but does not necessarily, progress to bloody diarrhoea within two to three days (Griffin 1995, Mead and Griffin 1998). Seventy percent or more of symptomatic patients develop bloody diarrhoea (Ostroff 1989; Bell 1994). Further manifestations of EHEC infection include hemorrhagic colitis (grossly bloody diarrhoea), haemolytic uremic syndrome (HUS),[1] and thrombotic thrombocytopenic purpura (TTP).

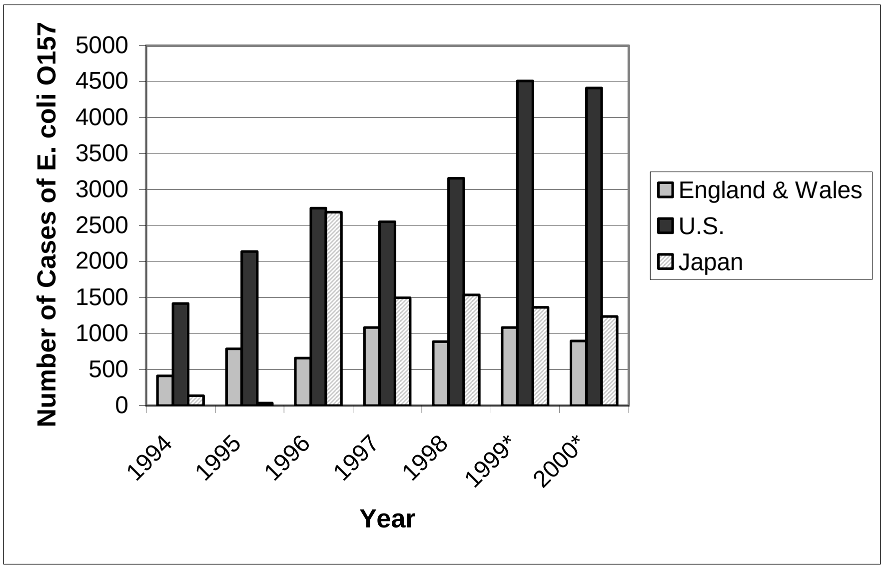
2.1.1 Susceptible populations

The incidence of EHEC infection varies by age group, with the highest incidence of reported cases occurring in children. In addition, the elderly are known to be susceptible to EHEC infection. However, persons of all ages can suffer infection from EHEC.

2.1.2 Annual incidence rate in humans including regional or seasonal variations in incidence or severity

During 1994-2000, the number of reported cases of *E. coli* O157:H7 infection in the U.S. increased more than two-fold from 1,420 (0.8/100,000 people) in 1994 to 4,410 (approximately 1.6/100,000 people) in 2000 (CDC 1999, CDC 2001) (Figure 1).

Figure 1. Number of reported cases of *E. coli* O157:H7 infection, U.S. (1994-2000),[a] England and Wales (1994-2000),[b] and Japan (1996-2000);[c] *provisional data are presented from 1999 and 2000 for the U.S.



a) CDC, NNDSS; Cases include suspect and confirmed human isolations.
b) PHLS Laboratory of Enteric Pathogens; Cases include only isolates, obtained from stool samples, that are submitted to PHLS from laboratories in England and Wales. They are confirmed, serotyped, phage typed and VT typed at PHLS.
c) Ministry of Health and Welfare, National Epidemiological Surveillance of Infectious Diseases; Cases are restricted to those with stool samples that have been culture confirmed and include all O157 serotypes.

[1] a combination of renal failure, low platelet counts and hemolytic anemia

In Belgium 97% of HUS cases in 2000 were associated with *E. coli* O157:H7 infection (Pierard et al. 1997). Siegler (1994) found that HUS caused chronic renal sequelae, usually mild, in 51% of survivors in Belgium (48% of all cases). Elliot (2001), however, has observed significantly lower renal failure statistics in Australia.

Between 1997 and 1999 at FoodNet sites located within the U.S., the overall incidence of HUS among children younger than 15 years of age was 0.7 per 100,000; this is similar to the frequency observed in other nations such as Austria (0.65 per 100,000) and Australia (0.64 per 100,000) (Elliot 2001). For children younger than 5 years of age, the incidence was 1.4 and 1.35 per 100,000 in the U.S. and Australia respectively (CDC 2000b). In a nationwide study of 83 patients with HUS in the U.S., 46 (55.4%) were younger than 5 years old and an additional 27 (32.5%) were 5 to 17 years old (Banatvala et al. 2001). Similarly, analyses of HUS incidence in Belgium found the majority (35/46) of HUS cases were in children (Pierard et al.1997). These findings suggest the burden of illness from HUS is comparable between Australia, North America and Europe. In addition, the strength of the association between HUS cases and EHEC infection appear similar in various parts of the world.

Interestingly, severity of EHEC infection has been shown to vary between sporadic cases and those associated with outbreaks. For example, between 3 and 7% of sporadic cases of *E. coli* O157:H7 infection progress to HUS, whereas 20% or more of outbreak associated cases progress to HUS (Mead and Griffin 1998). The reasons for this observation are unclear and as such merit further investigation.

3. Food production, processing, distribution and consumption

3.1 Source of contamination

EHEC strains have been isolated from gastrointestinal tracts and faeces of various domesticated and wild animals, including cattle, sheep, horses, pigs, turkeys, dogs, , seagulls and rats (Kudva 1996; Rice and Hancock 1995; Hancock et al.1998b; Heuvelink 1999), as well as from ill food handlers and human sewage. Foods associated either directly or indirectly with animals (meat or dairy products) or animal waste products (via application of fertilizer, for instance) have been frequently implicated as vehicles of transmission for EHEC infection. Data from epidemiological studies suggest cattle manure is an important source of EHEC infections. In fact, *E. coli* O157:H7 has been described as "ubiquitous" in dairy and beef cattle and is present at least occasionally on most farms or feedlots (Hancock et al. 1998a; Hancock et al. 2001).

Many of the risk factors that are thought to influence EHEC prevalence and their levels in cattle apply to whole herds rather than to individual cattle. Therefore, mitigation strategies for reduction of EHEC in agricultural settings typically target herd-level risk factors. The type of herd appears to have an effect on the prevalence of EHEC: herds of feedlot cattle (steers and heifers) are more likely to be colonized with EHEC than are breeding herds (cows and bulls). In addition, when a feedlot herd is positive for EHEC it is likely to have significantly more colonized animals than breeding cattle herds (USDA, 2001). Limited evidence suggests that dairy and cow-calf herds are similar to each other with respect to EHEC prevalence (Lagreid et al. 1999; Sargeant et al. 2000). An increased seasonal incidence of EHEC infections in cattle and human populations has been demonstrated in the warm months (Hancock et al. 1997a, 1997b; Griffin 1998; Van Donkersgoed et al. 1997, Heuvelink 1998). The roles that water effluent used to irrigate animal feed and crops, the age of the animals to which the feed is administered, and the feed itself play in colonizing herds may prove to be critical to on farm management strategies and should be further investigated (Anderson et al. 2001, Hancock et al. 2001, LeJeune et al. 2001). Similarly, the relative importance of food production worker health and hygiene as relates to EHEC contamination must be further elucidated.

3.2 Characteristics of the commodities

3.2.1 Leafy Green Vegetables

Leafy green vegetables grown low to the ground are a recognized cause of outbreaks of EHEC infection. Contamination of vegetables may occur in several ways, including through use of manure or water contaminated with fecal matter (Solomon et al. 2002a; Wachtel et al. 2002a; Solomon et al. 2002b). The fact that much produce is minimally processed and consumed raw increases the likelihood of EHEC infection. Use of good management practices (GMPs), such as those aimed at ensuring water quality, worker health and hygiene, pest control, and proper sanitation, provides the cornerstone for minimizing food safety hazards from EHEC in fresh vegetables and fruits (FDA 1998).

3.2.2 Beef

Meat becomes contaminated with EHEC when carcasses come into contact with faeces and/or contaminated hides during slaughter (Elder et al. 2000). Consequently, determination of the quantitative association between the incoming status of cattle and the outgoing status of harvested meat, in terms of EHEC contamination, is critical in an exposure assessment for EHEC infection. Such quantitative correlation between pre-harvest and post-harvest contamination is likely best predicted using fecal *E. coli* O157:H7 prevalence data (Elder et al. 2000).

3.3 Retail and Consumer Behaviour

The food preparation industry as well as consumer choices and behaviours have a large influence on the probability of contracting an EHEC infection. Specifically, undercooked beef (in particular ground or minced products) is correlated to infection, as previously discussed. (Although cooking such products to an internal temperature of ≥68 ˚C has been demonstrated an adequate precaution, consumers continue to choose undercooked beef products.)[2] Similarly, awareness of and precautions against cross-contamination between raw meat products and either cooked foods or raw vegetables would likely limit the likelihood of infection.[3] Consumer behaviour that can reduce illness from vegetables contaminated with EHEC at the farm is largely limited to thoroughly cleaning produce, in particular commodities to be consumed raw; however, this practice is not guaranteed to eliminate surface contamination by EHEC. Furthermore, recent research has demonstrated that sprouted seeds and some fruits and vegetables can internalize EHEC (Solomon et al. 2002a). In such instances, surface cleaning may not be sufficient for illness prevention.

3.4 Interventions

A recent Canadian risk assessment predicted the reduction in illnesses expected from various mitigation scenarios for ground beef (Table 3) (Cassin et al. 1998). These mitigations included achievement of maximum temperature control during storage, pre-slaughter screening of cattle faeces and cooking at appropriate temperatures. Based on the Cassin approach, an Australian risk assessment also modeled risk mitigation scenarios including hot water decontamination of carcasses, irradiation of frozen boxed beef, pre-slaughter reduction in faecal concentrations, retail temperature control and consumer education about good cooking practices (Lammerding et al. 1999; P. Vanderlinde, personal communication).

Due to the impact on-farm cattle colonization can have on other commodities, such as leafy green vegetables, interventions that control EHEC in live animals are of great interest in terms of curtailing EHEC infection. Potential interventions include employing probiotic bacterial flora in cattle (Zhao et al. 1998), implementing various feeding regimens (Cray et al. 1998), and using different composting and irrigation protocols (Lung et al. 2001).

EXISTING CODEX DOCUMENTS RELEVANT TO THE CONTROL OF EHEC IN VARIOUS COMMODITIES OF CONCERN

The Risk Management strategies for reducing human illness from EHEC due to consumption of leafy green vegetables and beef are as follows.

1. Leafy Green Vegetables

Bacterial contamination of commodities derived from fresh produce (e.g., sprouted seeds and prepared i.e., pre-cut and bagged, lettuce) may be due to several factors, including, for example: (1) poor irrigation practices, (2) inadequate cleaning, (3) cleaning with contaminated water, (4) non-hygienic farm workers, (5) wild and/or domestic animals, (6) wind transport of pathogens, and (7) use of manure as fertilizer. Existing international guidance directed toward the production of these products (Code of Hygienic Practice for Fresh Fruits and Vegetables, ALINORM 03/30, Appendix II) may mitigate and address the majority of the problems cited above, provided the CHPFFV is properly

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[2] http://www.fsis.usda.gov/oa/news/1998/colorpr.htm
[3] http://www.fsis.usda.gov/oa/pubs/keep_apart.htm

implemented and managed. Importantly, however, said guidance does not effectively address methods specifically targeting the reduction of human illness from EHEC associated with contamination from agricultural environments. Additionally, educational efforts may be most effective for reducing human illness from EHEC associated with cross contamination during food preparation.

2. Beef

Contamination of ground beef is usually a consequence of faecal contamination that occurs at, or is not adequately removed during slaughter. Current international guidance directed toward the production of hygienic meat includes the General Principles of Meat Hygiene[4] (under development) and the Code of Hygienic Practice for Fresh Meat[5] (also under development). These documents include useful approaches for minimizing contamination of beef such as implementation of HACCP and good plant sanitation. Additionally, they provide some guidance on the importance of:

- Feedstuffs in minimizing cattle colonization and potential impact of sub-therapeutic antibiotic use. (Footnote 2, Paragraphs 24, 25 and 27)
- Maintenance of animal hygiene throughout transport. (Footnote 2, Paragraphs 17, 30 and 40)
- Development of rigorous record keeping systems both on-farm and in-plant to facilitate animal identification and trace-back. (Footnote 2, Paragraphs 16, 38 and 46)
- Ensuring only clean and healthy animals are presented for slaughter through ante-mortem inspections and other means determined useful to the establishment. (Footnote 2, Paragraphs 34, 38 and 40)

Based on review of existing Codex risk management guidance information pertinent to the control of EHEC in foods, it appears appropriate for the Committee to consider developing a separate guidance document on methods for minimizing EHEC infection associated with the consumption of foods contaminated with *E. coli* O157:H7 from bovine faecal material. The Committee should consider whether the development of such a guidance document can best be accomplished after the Committee is further informed by a complete risk assessment or whether guidelines to reduce EHEC infections can be developed based on the information that is currently available.

RISK ASSESSMENT NEEDS AND QUESTIONS FOR THE RISK ASSESSORS

If a farm-to-table risk assessment for EHEC in ground beef and leafy green vegetables is deemed necessary, development of an on-farm module that could be used in combination with other modules for risk assessments of EHEC infection associated with either ground beef or leafy green vegetables should also be considered.

On-farm mitigation strategies that could be investigated by risk assessors for their possible effect on reducing human illness include:

- The effect of probiotics and bacteriophage (http://www.amif.org/ProbioticsReport042302.pdf)
- The effect of specific feeding regimens on minimizing fecal shedding of EHEC (Cray et al. 1998)
- The effect of specific manure composting regimens (Lung et al. 2001)
- The effect of distance and water treatment, distribution and cross-contamination on contamination of downstream and downwind crops by bovine faeces

Other mitigations (discussed in further detail in the appended risk profile) that could be evaluated in the risk assessment include:

- Measures used to prevent or control growth of EHEC in foods during transit and storage (temperature, antimicrobials, etc).
- Measures to minimize contamination of carcasses at slaughter

[4] Alinorm 03/16: Appendix II
[5] Alinorm 03/16: Appendix III

- Measures to minimize consumer exposure to contaminated products, including ground beef and leafy green vegetables (e.g., surveillance programs, consumer education programs, etc.)
- Other measures designed to minimize contamination of food products (e.g., Recommended Codes of Practice, Risk Management Guidelines, Certification Programs, etc.)

AVAILABLE INFORMATION

A number of countries have evaluated the risk associated with foodborne EHEC (Table 3). Specifically, Canada has analyzed the risk associated with *E. coli* O157:H7 infection from consuming ground beef hamburgers (Cassin 1998), sprouts (personal communication with Health Canada, January 2002) and juices (personal communication with Health Canada, January 2002), each of which have contributed to outbreaks or sporadic incidents of illness in that nation. An academic group in Canada has also assessed risk factors associated with on-farm *E. coli* O157 prevalence in cattle (Jordan 1999a, 1999b). The Netherlands chose to investigate steak tartare as the vehicle of transmission in their risk assessment because: (1) a steak tartare is thicker than a hamburger, and therefore the risk of insufficient heating of the center is larger, (2) people tend to accept a partially raw tartare but do not accept a partially raw hamburger, and (3) tartare is sometimes consumed raw (e.g., a tartare roll in snack bars). The U.S. has developed a farm-to-table risk assessment for *E. coli* O157:H7 in ground beef in addition to a comparative risk assessment for *E. coli* O157:H7 in tenderized and non-tenderized steaks. Due to the smaller contribution of *E. coli* O157 serotypes to human illness in Australia, that country has developed one risk assessment for O157:STEC and another for all STEC in ground beef production and in fermented meat. FAO/WHO may find many of these risk assessments useful in the development of a risk assessment for Codex. Further evaluation of each is warranted.

Table 3: Risk assessments for *E. coli* O157:H7

Nation	Topic	Reference
Australia	Ground Beef[1]	Lammerding 1999
Australia	STEC in Ground Beef[1]	Lammerding 1999
Canada	Ground Beef Hamburgers	Cassin 1998
Canada	Seeds/Beans and Sprouted Seeds/Beans[2,3]	Personal Communication with Health Canada
Canada	Unpasteurised Fruit Juice/Cider[4]	Personal Communication with Health Canada
Canada	Pre-harvest Husbandry Practices	Jordan 1999a,b
Ireland	Beef/Beef Products	www.science.ulst.ac.uk/food/E_coli_Risk_ Assess.htm
Netherlands	Raw Fermented Products	www.research.teagasc.ie/vteceurope/S+Gprog /hoornstrasg.html
Netherlands	Steak Tartare	RIVM report 257851003/2001
U.S.	Ground Beef[5]	www.fsis.usda.gov/OPPDE/rdad/FRPubs/00-023NReport.pdf
U.S.	Tenderized vs. Non-tenderized Beef Steaks	Personal Communication with USDA

[1]ANZFA Food Standard Code 1.6.1 sets Microbiological limits for total generic E. coli in a variety of foods. http://www.anzfa.gov.au/foodstandardscodecontents/standard16/index.cfm. Additionally, dairy products must be produced from pasteurized milk.

[2]Subsequent policy and management documents include “Consultation/Policy Document: A Dialogue on Developing a Risk Management Strategy for Sprouted Seeds and Beans”.

[3]Subsequent policy and management documents include “Code of Practice for the Hygienic Production of Sprouted Seeds”

[4]Subsequent policy and management documents include “Code of Practice for the Production and Distribution of Unpasteurised Apple and Other Fruit Juice/Cider in Canada”

[5]The U.S. has a microbiological criterion that requires absence of *E. coli* O157:H7 in raw ground beef

DATA GAPS

Several data gaps have been identified based on currently available risk assessments for *E. coli* O157:H7, including:

- Information describing the human health impact of EHEC in less developed nations
- Commodities likely to be associated with EHEC foodborne illness in less developed nations
- Data regarding the exposure dose of EHEC likely to cause illness in susceptible populations
- Frequency and severity of EHEC illness among children ages 0 to 5 years, in particular among those that become ill from consuming ground beef and raw produce
- Industry and consumer practices for various methods of manufacturing, cooking, and consuming ground beef and raw vegetables.
- Survival of EHEC on produce as a result of contamination by water or organic fertilizer
- Survival and spread of EHEC in the environment
- Information describing the critical contamination levels of meat products that may lead to cross contamination of uncooked produce
- Information on the incidence of fresh leafy vegetables contaminated by bovine faeces containing EHEC, as opposed to feral animal faeces or human faeces
- Information on the maximum density of EHEC in ground beef and raw vegetable servings as a result of matrix effects, competitive microflora, and various other environmental conditions (e.g., pH, water activity).
- Predictive microbiological data on the increase and decrease in the number of EHEC in ground beef and on raw vegetables under various storage and preparation conditions, together with frequencies of occurrence of these storage and preparation conditions
- Data on cross-contamination of EHEC between carcasses during carcass splitting
- Time-temperature data (quantitative) for chillers in slaughter establishments
- Marketing data on the proportion of beef ground at slaughter versus retail
- Data on retail and consumer storage, cooking, and consumption (frequency and serving size) patterns by type of ground beef meal (e.g., grilled hamburger in July vs. baked meat loaf in October).
- Descriptive epidemiologic information about sporadic cases of EHEC infection, including the month of disease onset, age and sex of victim, hospitalizations, summary of clinical manifestations (including severe disease manifestations), and food vehicles involved (if known)
- Additional case-control studies of sporadic EHEC infection so to calculate the etiologic fraction attributable to ground beef
- Information on processing and mitigation strategies for reducing EHEC in meat and vegetables

DRAFT RECOMMENDATIONS

- Determine whether the currently available information is sufficient for the development of guidelines for the control of EHEC in foods, including ground beef and leafy green vegetables.
- If a risk assessment is necessary, the Committee should consider the evaluation of an on-farm module that could be used to clearly answer questions related to the impact of various on-farm practices (e.g., manure control strategies) on the number of cases of human illness from EHEC associated with consumption of ground beef or leafy green vegetables

- After evaluating the currently available information and/or the outputs from the above risk assessment, the Committee should develop a separate guidance document on methods for minimizing EHEC infection associated with the consumption of foods contaminated with bovine faecal material.
- Along with developing the separate guidance document for the control of EHEC in foods, the Committee should re-evaluate existing Codex food codes and guidance documents to consider whether any should be amended or if annexes should be developed for them. This would require a thorough review of these documents in light of the risk assessment, if conducted. Examples include:
  - General Principles of Meat Hygiene[6] (under development)
  - Code of Hygienic Practice for Fresh Meat[7] (under development).
- Encourage research efforts to address the data gaps (listed previously) in order to develop more informed and appropriate risk management guidance
- Consider using existing national risk assessments for *E. coli* 0157:H7 to examine the predicted outcomes of the various risk management strategies that would be considered for the control of this hazard.

[6] Alinorm 03/16: Appendix II
[7] Alinorm 03/16: Appendix III

Appendix A

ENTEROHEMORRHAGIC *ESCHERICHIA COLI* (EHEC) INFECTION

REVISED RISK PROFILE

JANUARY 2004

1. PATHOGEN-FOOD COMMODITY COMBINATION(S) OF CONCERN

Escherichia coli

Escherichia coli strains that are pathogenic for humans may be categorized into specific groups based on virulence properties, mechanisms of pathogenicity, and clinical syndromes. These categories include enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), diffusely-adherent *E. coli* (DAEC), enteroaggregative *E. coli* (EaggEC), and enterohemorrhagic *E. coli* (EHEC). The EHEC group comprises a subset of Shiga toxin-producing *E. coli* (STEC),[8] which includes strains of *E. coli* that cause bloody diarrhoea. STEC produce either or both of two phage-encoded toxins, Shiga toxin 1 (Stx1) and Shiga toxin 2 (Stx2). However, Stx production in and of itself may not be enough to cause illness. Some EHEC strains also contain genes that encode for the ability to attach to and damage intestinal tract cells, causing what is commonly referred to as attaching-and-effacing lesions. For a detailed review of the pathogenesis of EHEC and other STEC, interested readers are referred to recent publications by Paton and Paton (1998) and Nataro and Kaper (1998).

1.1 Pathogen of concern

EHEC were first identified as human pathogens in 1982, when strains of a previously uncommon serotype, O157:H7, were implicated in two outbreaks of hemorrhagic colitis (bloody diarrhoea) in the United States (U.S.) (Riley et al. 1983; Wells et al. 1983). Since then, outbreaks due to this pathogen have become a serious public health problem throughout many regions of the world (Schlundt 2001; Clarke et al. 2002). Continued occurrence of large outbreaks and an increase in the incidence of reported cases suggests *E. coli* O157:H7 is a prototypical emerging pathogen (Tauxe 1997; Altekruse et al. 1997). In addition to *E. coli* O157:H7, in the 1990s, EHEC strains of other serogroups such as O26, O103, O111, and O145 were increasingly linked to human illness, as illustrated by surveillance data from Japan (Table 1). The WHO recently reported *E. coli* O26, O103, O111, and O145 are the most important non-O157 serogroups, in terms of public health (WHO 1998). At least three outbreaks in the U.S. have been ascribed to non-O157 EHEC: a familial outbreak of *E. coli* O111 with a case of HUS, a milk-associated episode of *E. coli* O104:H21 affecting 18 individuals, and an outbreak of gastrointestinal illness, including bloody diarrhoea, associated with *E. coli* O111:H8 in 56 persons (CDC 2000). Non-O157 serotypes of *E. coli* including O26:H11, O111:H8, O103:H2, O113:H21, and O104:H21 have been responsible for a small number of outbreaks in other parts of the world (CDC 1995b; Goldwater and Bettelheim 1995; Paton et al. 1996; Robins-Browne et al. 1998). Interestingly, in a cluster of three cases of HUS caused by O113:H21 in Australia, the causative organism was found not to possess the attaching-and-effacing gene (see above) (Paton et al. 1999). Furthermore, in countries such as Australia *E. coli* O157:H7 has not made a major impact, strains of serotypes O26 and O111:H- being of greater concern. However, these data notwithstanding, the preponderance of available data suggest *E. coli* strains of serotype O157 should continue to be considered the foremost concern for EHEC infection.

Table 1: Serotypes of human EHEC isolates from 1999-2000 in Japan*

Serotype	1999 Cases (% of total)	2000 Cases (% of total)
O157	1394 (72.1)	1158 (69.9)
O26	346 (17.9)	377 (22.8)
O111	81 (4.2)	42 (2.5)
All other	112 (5.8)	79 (4.8)

*http://idsc.nih.go.jp/iasr/22/256/graph/t2563.gif

[8] STEC are also referred to as VTEC (verotoxigenic *Escherichia coli*) in some member states. Both names are frequently employed in the scientific literature.

While *E. coli* O157:H7 are easily differentiated biochemically from other enteric *E. coli* because they ferment sorbitol slowly, diagnostic methods for identifying non-O157 EHEC are not widely available in most laboratories; consequently infections caused by these pathogens are often not confirmed. Recently, new methods for the detection of O26, O103, O111, and O145 serogroups have been developed; these advances may facilitate the collection of more data regarding the prevalence and significance of these serotypes as it pertains to human foodborne illness (Cudjoe 2001). Mead et al. (1999) have estimated the incidence of non-O157 EHEC is between 20 and 50% that of *E. coli* O157:H7 infection.

1.2 Commodities of Concern

In order to choose the most appropriate product to consider in this risk profile, the frequency with which various products have been implicated in causing EHEC was considered. To accomplish this, we evaluated available studies of sporadic cases of EHEC infection and outbreak investigation reports. Sporadic cases account for the majority of reported cases in a given year and therefore may be more representative of persons with EHEC infection. For example, 75% of reported cases in one region of the U.S. during 1991-97, and 83% of reported cases in another region of the U.S. during 1992-1999, were sporadic (OCD 1998, Proctor and Davis 2000). Food vehicles implicated most frequently have been raw or undercooked foods of bovine origin, especially undercooked hamburgers and unpasteurised milk. Nevertheless, an increasing number of outbreaks have been associated with the consumption of raw or minimally processed fruits and vegetables.

Foods of bovine origin

Case control studies of sporadic illness have described the association between ground beef consumption (in most cases, undercooked product) and EHEC infection (Table 2). Grinding meat introduces the pathogen into the interior of the meat; thus when ground beef is not heated to an appropriate internal temperature (e.g., > 68°C)[9] or when it is cooked unevenly, EHEC may survive. Moreover, in most countries, many thousands of pounds of meat trim from many carcasses are ground together; therefore, a small number of carcasses with EHEC can contaminate a large supply of ground beef. Additionally, contaminated beef may transfer EHEC to meat grinding equipment, which may later contaminate other lots of raw meat. Ground-beef products, therefore, pose a greater hazard than do intact cuts of meat. Dry fermented meats have also been implicated in reported outbreaks of EHEC infection (Tilden et al. 1996). A case-control study showed a relation between consumption of two sausages, mortadella (cooked) and teewurst (fermented, containing beef), and illness (Ammon et al. 1999).

Table 2: Case-control studies implicating ground beef in EHEC infection

Study Reference	Study Type	Finding
Slutsker 1998	Case-control, sporadic illness	Consumption of ground beef with "pink center" had 34% population attributable risk.
Mead et al. 1997	Case-control, sporadic illness	45% of ill persons consumed ground beef with "pink center" in the preceding week while only 33% of controls did the same.
Kassenborg 2001	Case-control, sporadic illness	Ground beef with "pink center" was a statistically significant risk factor while consumption of just ground beef was not.
MacDonald 1988	Prospective study	Rare ground beef was consumed more often by ill persons than healthy persons.
Le Saux et al. 1993	Case-control, sporadic illness	Consumption of undercooked ground beef had an attributable risk factor of 17%.

Outbreak investigations have also contributed significantly to our understanding of how EHEC is transmitted to humans. For example, ground beef was identified as the transmission source in seven of 13 (53.9%) outbreaks that occurred between 1982 and 1993 in the U.S. (Griffin 1995). Outbreaks have been attributed to foodborne, waterborne and person-to-person means of transmission.

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[9] Recommendations ranging between 68.3 and 71 °C have been made. In some cases these are associated with holding times at the specified temperature such as 15 seconds.

Beef was cited as the source of 46% of the foodborne outbreaks with a known vehicle of transmission in the U.S. during the years 1993 to 1999. Of the 21 beef-associated outbreaks that occurred during 1998-1999, ground beef was identified as the vehicle in 19 (Table 3). Five (26.3%) of the 19 ground beef/hamburger-associated outbreaks occurred in multiple states. Two outbreaks in 1999 were attributed to roast beef and one of these was a result of environmental contamination from manure in a pasture where a picnic was held.

Table 3: Food Vehicles Implicated in Outbreaks of *E. coli* O157:H7, U.S., 1998-2001[a]

Vehicle	1998	1999	2000	2001	Total
Ground beef/hamburger	10	9	4	4	27
Roast Beef	0	2	0	1	3
Combined green leafy vegetables	4	7	1	1	13
Salad	1	1	1	1	4
Coleslaw (cabbage)	2	1	0	0	3
Lettuce	1	3	0	0	4
Milk	2	0	0	0	2
Other	5	5	6	2	18
Total No. Outbreaks[a]	21	21	11	8	63

[a]Outbreaks for which food vehicles were known; outbreaks due to unknown vehicles were not included.

[b]Note that because 'Combined leafy green vegetables' are further grouped into three sub-categories, the numerical values for 'Total No. Outbreaks' are not the sum of the numerals in their respective columns.

Sources: CDC 1999b; CDC 2001c.

Other products of bovine origin that have been implicated in a number of outbreaks of EHEC infection include raw and improperly pasteurised cow's milk, as was demonstrated by an *E. coli* O104:H21 outbreak from contaminated milk (Feng et al. 2001). Milkborne outbreaks mostly have been associated with the consumption of raw milk or milk products from local farms. Raw milk is often contaminated with enteric organisms during its collection and may result in a direct risk for consumers choosing to drink raw milk. It should be emphasized, however, effective pasteurisation eliminates pathogens from milk, including EHEC.

Foods of non-bovine origin

A variety of foods may become contaminated with EHEC through cross-contamination with beef or other meats and contaminated kitchen surfaces during food preparation. For instance, mayonnaise and mayonnaise-based dressings and sauces were identified as the most likely foods to have been contaminated in a recent series of outbreaks of *E. coli* O157:H7 infection in the U.S. (Jackson et al. 2000). Studies have indicated mayonnaise can serve as a vehicle for EHEC when stored at refrigeration temperatures, despite its relatively low pH. Sandwiches were cited as the probable source in an outbreak of *E. coli* O157:H7 at a nursing home (Carter et al. 1987). Additionally, several outbreaks associated with wild game meat have been reported (Asakura et al. 1998, Keene et al. 1997).

In addition, fruits and vegetables contaminated with EHEC have accounted for a growing number of recognised outbreaks (Table 3). Examples of vegetables, fruits, and sprouts implicated in foodborne outbreaks of EHEC infection include fresh potatoes (Morgan et al. 1988), lettuce (Ackers et al. 1998, Mermin et al. 1997, Hilborn et al. 1999), radishes (Michino et al. 1998), alfalfa sprouts (Breuer et al. 2001, MMWR 1997a), and cantaloupe (Del Rosario and Beuchat 1995). As a whole, leafy green vegetables were cited as the source of 26% of foodborne outbreaks with a known vehicle of transmission in the U.S. during the years 1998 to 99. Contamination of vegetables may occur in several ways, including use of manure or water contaminated with faecal matter as fertilizer (Solomon et al. 2002a; Wachtel et al. 2002a; Solomon et al. 2002b) and through handling by workers with poor health and hygiene. In a number of the instances cited above, manure from nearby cattle lots was suspected to be the original source of EHEC (Ackers et al. 1998; Hilborn et al. 1999). Similarly, in an accidental release of tertiary-treated sewage that had not been treated with chlorine, cabbage plants were found to have *E. coli* strains (not containing *stx1*, *stx2*, or *eae* genes) associated with the plant roots when control fields did not (Wachtel et al. 2002a). Another means of contamination of these products is cross-contamination in the retail, or consumer kitchen between contaminated meat products and produce.

Fruit juices have also been implicated in outbreaks of EHEC infection (Besser et al. 1993, CDC 1996, CDC 1997, Cody et al. 1999, MMWR 1997b, Steele et al. 1982). Although the low pH of fruit juices will generally not allow survival and outgrowth of many of the Enterobacteriaceae, they may allow survival of *E. coli* O157:H7 because of its high acid-tolerance. Although the exact mechanisms of contamination for these outbreaks were not clearly determined, animal manure was suspected to have contaminated the fruit.

In summary, there are many foodborne pathways by which individuals can be exposed to EHEC. Other important risk factors for contracting EHEC infection are exposure to farm animals or the farm environment, eating at a table service restaurant, using immune suppressive medication (for adults only), and obtaining beef through a private slaughter arrangement (Kassenborg et al. 2001; OCD 1998). Current data based on outbreaks and sporadic infections indicate consumption of ground beef remains the single most important source of foodborne EHEC infection. Leafy green vegetables are the second most significant cause of human foodborne illness cases of EHEC as they are subject to contamination and they are eaten raw. Table 5 in the final section of this document describes the national and academic risk assessments that have been conducted to date for *E. coli* O157:H7 infection.

Due to their relevance to human cases of EHEC infection, ground beef and green leafy vegetables are the focus of this risk profile. Commodities worthy of future consideration include raw milk products, unpasteurised cider, fresh-cut fruits, fermented raw meat products and sprouted seeds.

- Foods associated either directly or indirectly with animals (meat or dairy products) or foods subject to contamination by animal waste products such as fertilizer or agricultural runoff are frequently implicated as vehicles of transmission for human illness.
- Beef was cited as the source of 46% of the foodborne outbreaks with a known vehicle of transmission in the U.S. during the years 1993 to 1999.
- Leafy green vegetables were cited as the source of 26% of the foodborne outbreaks with a known vehicle of transmission in the U.S. for the years 1998 to 1999.
- Ground-beef products pose a greater hazard than intact cuts of meat.
- For these reasons, this working group will only address the threat associated with ground beef and leafy green vegetables that have been contaminated with bovine faeces as vehicles of transmission.

2. Description of the public health problem

Epidemiology

Following ingestion of EHEC, the human response ranges from asymptomatic infection to death. To cause disease after ingestion, EHEC must survive acidic conditions within the stomach prior to moving to distal portions of the gastrointestinal tract. Disease due to EHEC occurs primarily in the colon. The incubation period from the time of ingestion to the first symptoms ranges from one to eight days. Asymptomatic shedding of EHEC has been documented (Swerdlow and Griffin 1997); yet the proportion of exposed individuals who shed EHEC but do not develop symptoms is unknown. Typically the illness begins with abdominal cramps and nonbloody diarrhoea that can, but does not necessarily, progress to bloody diarrhoea within two to three days (Griffin 1995, Mead et al. 1998). Usually 70% or more of symptomatic patients will develop bloody diarrhoea (Ostroff et al. 1989; Bell et al. 1994). More severe manifestations of EHEC infection include hemorrhagic colitis (grossly bloody diarrhoea), haemolytic uremic syndrome (HUS),[10] and occasionally, thrombotic thrombocytopenic purpura (TTP).

Symptoms of hemorrhagic colitis include severe abdominal cramps followed by grossly bloody diarrhoea and edema (swelling), erosion, or haemorrhage of the mucosal lining of the colon (Su and Brandt 1995). Hemorrhagic colitis may be the only manifestation of EHEC infection, or it may

[10] a combination of renal failure, low platelet counts and hemolytic anemia

precede development of HUS. Complications from hemorrhagic colitis associated with EHEC include upper-gastrointestinal bleeding and stroke (Su and Brandt 1995). Roberts et al. (1998, citing Boyce et al. 1995a, Ryan et al. 1986) estimates the mortality rate of those suffering hemorrhagic colitis without progression to HUS to be 1%. Approximately 30 to 45% of patients are hospitalized (Ostroff et al. 1989, Le Saux et al. 1993, Bell et al. 1994, Slutsker et al. 1998). Of the 631 cases reported to FoodNet sites in 1999, 39% were hospitalized (CDC 2000b). Treatment for the more serious manifestations of EHEC infection is supportive and the use of antimicrobial agents has been debated (Mead and Griffin 1998).

The incidence of EHEC infection varies by age group, with the highest incidence of reported cases occurring in children. In addition to children, elderly are known to be susceptible to EHEC infection. A report detailing a Scottish outbreak resulting from contaminated beef involving at least 292 confirmed cases of EHEC infection resulted in 151 hospitalizations and 18 deaths (in which all fatalities were elderly patients) (Ahiiied 1997).

The number of reported EHEC cases derived from surveillance is known to underreport the true disease burden. Nonetheless, using surveillance data, and accounting for the factors that contribute to underreporting, Mead et al. (1999) estimated that 73,480 cases of *E. coli* O157:H7 infection occur annually in the U.S. and that 85% (62,456 cases) are a result of foodborne exposure. *E. coli* O157:H7 was designated by the Council of State and Territorial Epidemiologists as a nationally notifiable disease in the U.S. beginning in 1994. During 1994-2000, the number of reported cases of *E. coli* O157:H7 in the U.S. increased more than two-fold from 1,420 (0.8/100,000 people) in 1994 to 4410 (approximately 1.6/100,000 people) in 2000 (CDC 1999, CDC 2001) (Figure 1). Cases in the U.S. are reported by passive surveillance through the National Notifiable Diseases Surveillance System (NNDSS).[11] This is a passive surveillance system in which health care providers report notifiable disease cases to local or state health departments. Other national or regional surveillance systems include (1) Enter-net[12] which includes a catchment area of 15 European Union (EU) member states as well as Switzerland and Norway, (2) The Communicable Disease Network Australia – National Notifiable Surveillance System,[13] (3) Japan's Statistics on Communicable Diseases in Japan (former Ministry of Health and Welfare) and the National Epidemiological Surveillance of Infectious Diseases (NESID) which are reported in Infectious Agents Surveillance Reports[14] and (4) the EU's Zoonoses Reporting System.[15] In addition to these surveillance systems, the EU, Japan and U.S. have each developed a pulsed-field gel electrophoresis (PFGE) database to assist in epidemiological investigations of disease from this and other bacteria. The increase in reported cases of *E. coli* O157:H7 over time is probably due to a combination of factors, including (1) improvement in the effectiveness of the surveillance system over time, (2) increased awareness of *E. coli* O157:H7 infection among health care providers and the public leading to improved detection and reporting, (3) enhanced ability to detect disease through better diagnostic tests (see text box), and (4) a true increase in the incidence of disease. Figure 1 illustrates the rising incidence of *E. coli* O157:H7 infection in three different regions of the world. For more information on the global impact of EHEC see the WHO Consultation report (WHO 1997).

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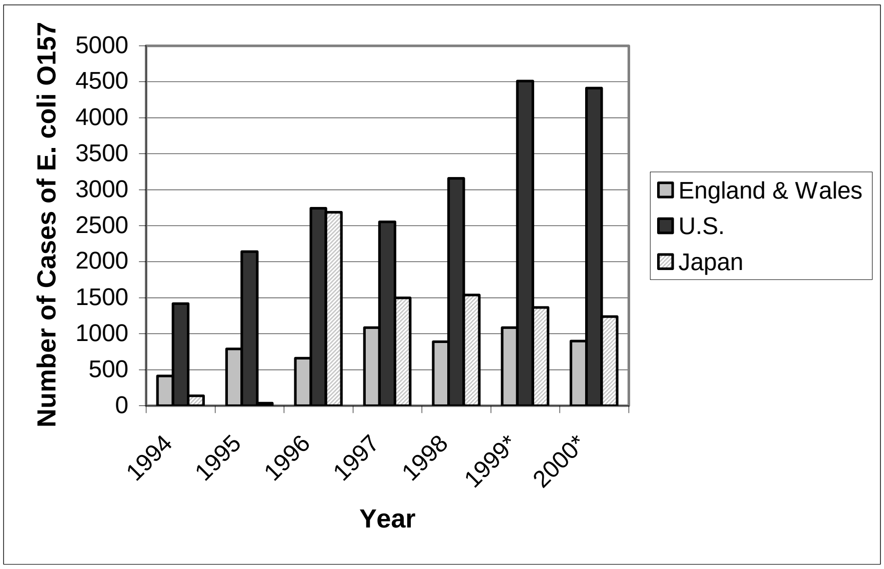
[11] http://www.cste.org/nndss/reportingrequirements.htm
[12] http://www.phls.org.uk/topics_az/ecoli/data.htm
[13] http://www.health.gov.au/pubhlth/cdi/nndss/year054.htm
[14] http://idsc.nih.go.jp/iasr/22/256/tpc256.html; http://idsc.nih.go.jp/index.html; Note that in the former system, known as the Ministry of Health and Welfare, communicable diseases in Japan were reported in "Statistics on Communicable Diseases in Japan" and, during a transitional period, in the "Annual Report on National Epidemiological Surveillance of Infectious Diseases". The new system, known as the National Epidemiological Surveillance of Infectious Diseases (NESID), publishes "Infectious Agents Surveillance Reports" monthly describing pathogen isolates and related information and "Infectious Disease Surveillance Data" annually describing notified human cases (IDSD is currently only available by CD-ROM format).
[15] EU Council Directive 92/117/EEC; http://europa.eu.int/eur-lex/en/com/pdf/2001/en_501PC0452_01.pdf

Figure 1. Number of reported cases of *E. coli* O157:H7 infection, U.S. (1994-2000),[a] England and Wales (1994-2000),[b] and Japan (1996-2000),[c] *provisional data are presented from 1999 and 2000 for the U.S.



a) CDC, NNDSS; Cases include suspect and confirmed human isolations.

b) PHLS Laboratory of Enteric Pathogens; Cases include only isolates, obtained from stool samples, that are submitted to PHLS from laboratories in England and Wales. They are confirmed, serotyped, phage typed and VT typed at PHLS.

c) Ministry of Health and Welfare, National Epidemiological Surveillance of Infectious Diseases; Cases are restricted to those with stool samples that have been culture confirmed and include all O157 serotypes.

In 1996, the Emerging Infection Program Foodborne Diseases Active Surveillance Network (FoodNet) began a program of active surveillance of clinical laboratories for specific foodborne diseases, including *E. coli* O157:H7. Five states in the U.S. participated initially (Minnesota, Oregon, selected counties of California, Connecticut and Georgia) (CDC 2001a). As of 2000, the areas under active surveillance included 8 states representing 29.5 million persons (10.8% of the 1999 U.S. population). The number of cases of *E. coli* O157:H7 infection reported annually to FoodNet ranged from 388 in 1996 to 631 in 2000 (Bender et al. 2000, CDC 2000b, CDC 2001a). Because the population under surveillance has increased, it is more appropriate to compare the number of reported cases per 100,000 persons in a population.

Data on the prevalence of symptomatic *E. coli* O157:H7 infection prior to the inception of FoodNet are scarce and include studies which estimate between two and 10 cases for every 100,000 persons (Ostroff et al. 1989, MacDonald et al. 1988). The higher estimates obtained in some of these studies is likely a consequence of the active method for data collection and may provide a more accurate estimate of the incidence of *E. coli* O157:H7 infection thereby suggesting that state-wide passive surveillance programs are hindered by underreporting.

HUS is the most common cause of acute renal failure in young children, yet it also has long-term complications. In Belgium 97% of HUS cases in 2000 were associated with *E. coli* O157:H7 infection (Pierard et al. 1997). Siegler et al. (1994) found that HUS causes chronic renal sequelae, usually mild, in 51% of survivors (48% of all cases) however, Elliot et al. (2001) have observed significantly lower renal failure statistics in Australia. Neurological complications occur in about 25% of HUS patients (Mead et al. 1998). Generally neurological symptoms are mild, but serious complications, such as seizure, stroke and coma, can occur (Su and Brandt 1995). Similar to treatment for EHEC infection, only symptomatic treatment for neurological complications is available, making this manifestation of HUS especially dangerous and an important cause of death in HUS patients. Other complications of HUS include pancreatitis, diabetes mellitus and pleural and pericardial effusions (Mead et al. 1998). In a nationwide study of HUS patients, 46 (55%) of 83 patients required either peritoneal dialysis or haemodialysis during the acute phase of their illness (Banatvala et al. 2001). Siegler et al. (1994) found that severe kidney or neurological impairments (end stage renal disease or stroke) occurred in 9

(5.7%) of *E. coli* 157:H7 HUS cases over a 20-year period in Utah. A number of studies have suggested the mortality rate associated with HUS is between 3 and 7% (Martin et al. 1990; Tarr and Hickman 1987; Rowe 1991 et al.; Mahon et al. 1997; Banatvala et al. 2001; Siegler et al. 1994).

The percent of *E. coli* O157:H7 infections which progress to HUS varies between sporadic cases and those associated with outbreaks. Between 3 to 7% of sporadic, and 20% or more of outbreak associated cases of *E. coli* O157:H7 infection will progress to HUS (Mead and Griffin 1998). The proportion of patients who develop HUS following *E. coli* O157:H7 infection is influenced by a variety of factors including age, bloody diarrhoea, fever, elevated leukocyte count, and toxin type (Griffin 1995). Wong et al. (2000) found that 10 (14.1%) of 71 children with *E. coli* O157:H7 infection developed HUS. Similarly, the severity of HUS illness may differ between sporadic cases and those associated with outbreaks; outbreaks often resulted in a shorter diarrhoeal prodrome, a higher rate of bloody diarrhoea and severe hemorrhagic colitis (Elliot et al. 2001).

Between 1997 and 1999 at FoodNet sites located within the U.S., the overall incidence of HUS among children younger than 15 years of age was 0.7 per 100,000; this was similar to the frequency observed in other nations such as Austria (0.65 per 100,000) and Australia (0.64 per 100,000) (Elliot et al. 2001). For children younger than 5, the incidence was 1.4 and 1.35 per 100,000 in the U.S. and Australia respectively (CDC 2000b). In a nationwide study of 83 patients with HUS in the U.S., 46 (55.4%) were younger than 5 years old and an additional 27 (32.5%) were 5 to 17 years old (Banatvala et al. 2001). In 1999, 35.3% of reported HUS cases in the U.S. occurred in 1- to 10-year-olds, 17.6% of cases occurred in 10- to 20-year-olds, and 14.1% of cases occurred in persons older than 60 (CDC 2000b). Similarly, analyses of HUS incidence in Belgium found the majority (35/46) of HUS cases were in children (Pierard et al. 1997). The overall findings of this study demonstrated that the burden of illness from HUS is comparable between Australia, North America and Europe. A national study of postdiarrheal HUS in the U.S. estimated that $\leq$ 20% of HUS cases were due to non-O157 EHEC; however, the authors qualified that estimate, commenting that it was difficult to determine the proportion of EHEC-associated HUS due to non-O157 EHEC (Banatvala et al. 2001). In Australia, between July 1994 and June 1998, only 8% of the EHEC associated cases of HUS were the result of *E. coli* O157 infection (Elliot et al. 2001). This suggests that while illness from HUS is similar on different continents, the predominant EHEC serotype responsible may vary.

Occasionally, patients with EHEC infection develop thrombotic thrombocytopenic purpura (TTP), a condition similar to HUS but one which is more likely to occur in adults and with more prominent neurological findings and less renal involvement than HUS. In a study by Banatvala et al. (2001), of 73 children and 10 adults that met the case definition of HUS, 8 (11.0%) children and 8 (80.0%) adults also met the case definition for TTP. None of the 8 children, but 2 (25.0%) of the adults died. That said, it should also be noted that there are causes of TTP other than the association with EHEC, and that prior to the 1980s, gastrointestinal infections were not strongly implicated in the pathogenesis of TTP (CDC 1986). Indeed, some evidence suggests that when associated with EHEC infection, TTP is probably the same disorder as HUS (Mead and Griffin 1998).

While the incidence of HUS is similar on different continents, the EHEC serotypes responsible for the syndrome may vary. Serotype O157:H7 however, remains as the prototypic EHEC strain and responsible for the majority of EHEC infections, as well as the leading cause of HUS worldwide. A number of national and regional disease surveillance systems record *E. coli* O157:H7 infections. Recently, the U.S. CDC has also included all Shiga toxin-producing *E. coli* on their surveillance list. A critical part of that effort is detection and improved diagnostic tests, such as polymerase chain reaction (PCR) assays for Shiga toxin genes has augmented our ability to detect EHEC in environmental samples, foods and water. Such assays offer quicker turn-around time and improved sensitivity, however, it is important to bear in mind that the implementation of new diagnostic tests may give rise to other complications (e.g., inability to compare incidence data generated by culture-based versus PCR-methods). Also, the financial costs of equipment, reagents and personnel training required to implement newer diagnostic technologies may render them impractical for developing countries.

3. Food production, processing, distribution and consumption

1. The farm to table continuum

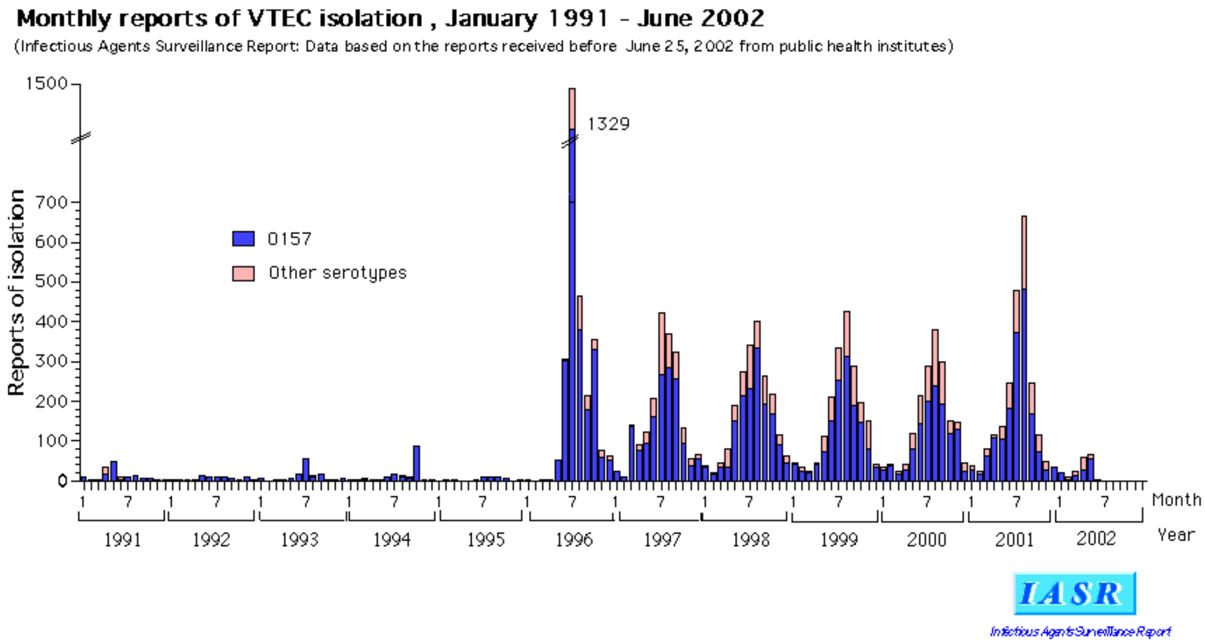
As was previously mentioned, EHEC have been isolated from the faeces or gastrointestinal tracts of cattle, sheep, horses, pigs, turkeys, dogs, and a variety of wild animal species (Kudva et al 1996; Rice and Hancock 1995; Hancock et al. 1998b; Heuvelink et al. 1999); consequently, foods associated either directly or indirectly with animals (meat or dairy products) or foods subject to contamination by animal waste products (for instance, via manure fertilizers) are frequently implicated as vehicles of transmission for human illness. Epidemiological studies have found that cattle manure is the primary source of most human *E. coli* O157:H7 infections. In fact, *E. coli* O157:H7 has been described as "ubiquitous" in dairy and beef cattle and is present at least occasionally on most farms or feedlots (Hancock et al. 1998a; Hancock et al. 2001). Factors contributing to the presence of *E. coli* O157:H7 in cattle include:

- the pathogen's ability to survive for at least 4 months in water trough sediments (Hancock et al. 1998a); and
- the pathogen's presence in animal feeds (Hancock et al. 2001).

Many of the risk factors that are thought to influence EHEC prevalence and their levels in cattle apply to whole herds rather than to individual cattle. Therefore, mitigation strategies typically target herd-level risk factors for EHEC control. The roles that water, including effluent used to irrigate animal feed and crops, age of the animals receiving feed, and the feed itself play in colonizing herds may prove to be critical to on farm management strategies and should be considered (Anderson et al. 2001, Hancock et al. 2001, LeJeune et al. 2001). Herds of feedlot cattle (steers and heifers) are more likely to have colonized animals than breeding herds (cows and bulls). Additionally, when a feedlot herd is positive for EHEC it is likely to have significantly more colonized animals than breeding cattle herds (USDA, 2001). Limited evidence suggests dairy and cow-calf herds are similar with respect to *E. coli* O157:H7 (Sargeant et al. 2000).

An increased seasonal incidence of *E. coli* O157:H7 infections in cattle and human populations has been demonstrated in the warmer months of the year (Hancock et al. 1998a, 1998b; Heuvelink et al. 1998). The same trend has been observed based on data from Japan (Figure 2). It is therefore not surprising that the incidence of HUS is also more common in the summer months (www.hcsc.gc.ca/hpb/lcdc/bmb/epiic95/95_ii_e.html; Mead and Griffin 1998; CDC 1999a; CDC 2000b, Van de Kar 1996). Of the sporadic cases of human *E. coli* O157:H7 infection reported by FoodNet sites for the years 1996 to 1998, 70% occurred during the months of June through September (Bender et al. 2000; CDC 1999a).

Figure 2: From IASR Infectious Agents Surveillance Report at http://idsc.nih.go.jp/prompt/graph/vt9.gif



Characteristics of the commodities

Leafy green vegetables grown low to the ground are a recognized cause of EHEC outbreaks. Contamination of vegetables may occur in several ways, including through application of manure or water contaminated with fecal matter (Solomon et al. 2002a; Wachtel et al. 2002a; Solomon et al. 2002b; Wachtel et al. 2002b). The fact that fresh produce is minimally processed and consumed raw increases the likelihood of EHEC infection. Use of good management practices (GMPs), such as those aimed at ensuring water quality, worker health and hygiene, pest control, and proper sanitation, provides the cornerstone for minimizing food safety hazards from EHEC in fresh vegetables and fruits (FDA 1998).

Meat becomes contaminated with EHEC when beef carcasses come into contact with contaminated hides and faeces during the slaughter process (Elder et al. 2000). Thus, a determination of the quantitative association between the incoming status of cattle and the outgoing status of harvested meat is critical in an exposure assessment. This quantitative correlation between pre-harvest and post-harvest contamination may best be predicted using fecal *E. coli* O157:H7 prevalence data (Elder et al. 2000).

A number of factors have a significant influence on the survival and growth of EHEC in food, including temperature, pH, salt, and water activity (Meng and Doyle 1998). Studies on the thermal sensitivity of *E. coli* O157:H7 in ground beef indicate the pathogen has no unusual resistance to heat and that heating ground beef sufficiently to kill typical strains of *Salmonella* will also kill *E. coli* O157:H7. The optimal temperature for growth of *E. coli* O157:H7 is approximately 37°C (98.6°F), and the organism will not grow at temperatures below 8°C to 10°C (46°F to 50°F) or above 44°C to 45°C (Doyle and Schoeni 1984; Buchanan and Doyle 1997). *E. coli* O157:H7 survives freezing, with some decline in the concentration of *E. coli* O157:H7 (Ansay et al. 1999).

E. coli O157:H7 has been reported to be more acid-resistant than other *E. coli*. Acid resistance may thus enhance the survival of EHEC in mildly acidic foods and may thus explain their ability to survive passage through the stomach. However, the extent of acid-resistance varies among EHEC strains and is influenced by growth phase and other environmental factors. Once induced, acid resistance is maintained for long periods of time during cold storage (Meng and Doyle 1998). Stationary-phase *E. coli* O157:H7 are more resistant than growing cells to acid (Meng and Doyle 1998). The presence of other environmental stresses, such as temperature or water activity stress will raise the minimum pH for growth (Buchanan and Doyle 1997). *E. coli* O157:H7 survives in such foods as dry salami, apple cider, and mayonnaise, which were previously considered too acidic to support the survival of foodborne pathogens. The literature contains conflicting reports about the efficacy of acid spray washing of beef carcasses for reduction of EHEC. A study by Brackett et al. (1994) found that warm and hot acid sprays did not significantly reduce the concentration of *E. coli* O157:H7 on beef carcasses. On the other hand, two recent studies have found organic acids to be effective in reducing the presence of *E. coli* O157:H7 on beef carcasses (Berry and Cutter 2000; Castillo et al. 2001). These results may reflect differences in acid resistance among strains of *E. coli* O157:H7 (Berry and Cutter 2000).

E. coli O157:H7 can survive for extended periods under conditions of reduced water activity while refrigerated; however, the organism does not tolerate high salt conditions (Buchanan and Doyle 1997).

Retail and Consumer Behavior

The food preparation industry and consumer choices and behaviours have a large influence on the probability of contracting an EHEC infection. Specifically, undercooked beef (in particular ground or minced products) is correlated to risk of infection (see above). Interestingly, although cooking beef products to an internal temperature of ≥68 ˚C has been shown to be an adequate precaution against EHEC infection, consumers continue to choose undercooked beef products.[16] In addition, in certain countries consumption of products made from raw minced beef (such as "tartare" steak, beef "americaine") is common. Furthermore, awareness of and precautions against cross-contamination between raw meat products and either cooked foods or raw vegetables would likely reduce the likelihood of infection.[17] Consumer behaviour that can limit illness from vegetables contaminated at the farm is likely limited to thoroughly cleaning produce, in particular those commodities destined to be consumed raw. Nonetheless, in the case of sprouted seeds and some fruits and vegetables which have been shown to internalize EHEC (Solomon et al. 2002a), washing may not be a sufficient intervention.

Interventions

A Canadian risk assessment was published that predicted the reduction in illnesses to be expected from various mitigation scenarios for ground beef (Table 4) (Cassin et al. 1998). These mitigations included achievement of maximum temperature control during storage, preslaughter screening of cattle faeces and cooking at appropriate temperatures. Based on the Cassin approach, an Australian risk assessment also modeled risk mitigation scenarios including hot water decontamination of carcasses, irradiation of frozen boxed beef, preslaughter reduction in faecal concentrations, retail temperature control, and consumer education about good cooking practices (Lammerding et al. 1999). To summarize:

- Meat becomes contaminated with *E. coli* O157:H7 when beef carcasses come into contact with contaminated hides and faeces during the slaughter process.
- An increased seasonal incidence of *E. coli* O157:H7 infections in cattle and human populations has been demonstrated in the warmer months of the year.
- *E. coli* O157:H7 has no unusual resistance to heat and heating ground beef sufficiently to kill typical strains of *Salmonella* will also kill *E. coli* O157:H7.
- Consumer choices and actions such as undercooking beef have a large influence on the probability of contracting an *E. coli* O157:H7 infection.

Due to the impact on-farm cattle colonization can have on other commodities such as leafy green vegetables, interventions that control EHEC in farm animals are of great interest. These include the impact of employing probiotic (Zhao et al. 1998) bacterial flora in cattle, the impact of various feeding regimens (Lung et al. 2001), the result of different composting protocols, (Cray et al. 1998) and the impact of various irrigation methods.

[16] http://www.fsis.usda.gov/oa/news/1998/colorpr.htm
[17] http://www.fsis.usda.gov/oa/pubs/keep_apart.htm

4. Risk Assessment Needs and Questions for the Risk Assessors

Is a microbiological risk assessment appropriate to fulfil the desired CCFH output(s)?

As both leafy green vegetables grown low to the ground and ground beef have been commonly associated with EHEC infections, they should each be considered, either together or separately, for inclusion in any risk assessment that is commissioned. Other products that have been associated with EHEC infections such as unpasteurised milk products, unpasteurised fruit juices and fermented uncooked meat products should also be considered for inclusion in a risk assessment. It should be recognized that the Committee may find that sufficient information exists for the development of risk management guidelines without the need for a quantitative risk assessment.

CCFH should encourage the implementation of practices that can be used to prevent or minimize (1) the colonization of cattle with EHEC, (2) the contamination of ground beef with faeces (3) the contamination of water with bovine faeces and (4) the contamination of food crops with bovine faeces. It is possible that the benefits achievable through downstream interventions are less important than those obtained via interventions at the farm, due in large part to the multiple infection pathways (and commodities) that can be minimized through effective manure management on the farm. A risk assessment may be useful in evaluating risk management options within the context of a farm-to-table continuum so that their relative importance can be definitively established.

If a risk assessment is undertaken by FAO/WHO, it should provide an estimate of the risk of illness and death from EHEC in those countries for which sufficient data are available. In so doing, illness should be explicitly defined by CCFH as a specified endpoint. Also, an assessment of the potential benefit (i.e. number of cases of illness and death prevented) for risk management strategies including those currently in effect or under consideration in member states (listed below) may be useful.

Measure effect of controlling EHEC at the farm level with respect to subsequent agricultural use of manure.

- The effect of employing probiotic bacterial flora in cattle.
- The effect of various feeding regimens.
- Outcomes from different composting protocols
- The effect of various irrigation methods (Solomon et al. 2002a)
- The effect of proper and effective water treatment and application during processing
- The effect of hygienic measures at the farm (such as cleaning and disinfection of premises between lots)
- Calf management at weaning

Measures to minimize faecal shedding of EHEC in animals presented for slaughter (Belgium, Sweden, others)

- Enforcing or tightening controls on animal feed quality.
- The effect of employing probiotic bacterial flora in cattle.
- The effect of various feeding regimens and fasting before transport
- Calf management at weaning
- Monitoring of human illness with mandatory testing of farms linked to an outbreak of human illness resulting from EHEC. Restrictions on positive farms include controls over the sale of live animals, and restriction on the sale of animals for slaughter (slaughter hygiene and swab tests at slaughter).

Measures to minimize contamination of carcasses at slaughter (Australia, Denmark, Germany, Portugal, Sweden, U.K., U.S.).

- Scoring the level of faecal contamination on the hides of incoming animals with remedial action (e.g. logistic slaughter) when scores exceed a predetermined level (clean cattle policy).
- Rodding and clipping the oesophagus and bagging and tying the rectum.

- Hygienic dressing and evisceration.
- Random testing of *E. coli* O157:H7 on beef carcasses in the slaughterhouse. A positive test results in testing on the farm of origin. Positive on-farm tests resulting in increased farm sanitation measures.
- Random testing of carcasses for (generic) *E. coli* contamination and measures to improve sanitation when predetermined levels are exceeded.
- Random testing of carcasses for enterobacteriaceae.
- Visual inspections of carcasses.
- The use of HACCP in slaughter and processing.
- Different decontamination procedures.

Measures to minimize consumer exposure to contaminated products (Australia, U.K., U.S.).

- Random testing for *E. coli* O157:H7 in meat (trim and ground) and on produce.
- Random testing for (generic) *E. coli* or enterobacteriaceae in meat (trim and ground) and on produce.
- Destruction or diversion of *E. coli*-positive meat product to cooked product.
- Irradiation or pasteurization.
- Specified cooling guidance.
- Enhanced hygienic practice during cutting, boning and other steps between slaughter and retail.
- Restaurant cooking requirements.
- HACCP in the food production and service sector.

Retail Codes / Consumer education

Cooking ground beef to a specified internal temperature as indicated by the use of a meat thermometer (Canada, Germany, U.S.)

Measures to minimize contamination of food products in international trade (special consideration of CCFH)

A certification program to ensure that exported products meet the acceptable level of protection of the importing country.

A WHO Consultation discussed the global impact of EHEC and the controls and prevention strategies employed by a number of nations (WHO 1997). European national guidance documents and mitigations are discussed in the European Commission Health and Consumer Protection Directorate-General's report SANCO/4320/2001.[18] Canada has produced (1) interim guidelines for the control of STEC, including *E. coli* O157:H7, in ready-to-eat fermented sausages containing beef or a beef product,[19] (2) policies concerning raw products of animal origin,[20] and (3) policies for ground beef containing *E. coli* O157:H7.[21] Australia has developed microbiological standards and Advisory

[18] http://europa.eu.int/comm/food/fs/inspections/special_reports/sr_rep_4320-2001_en.pdf
[19]http://www.hc-sc.gc.ca/food-aliment/english/organization/microbial_hazards/guideline_for_fermented_sausages.html
[20]http://www.hc-sc.gc.ca/food-aliment/english/organization/microbial_hazards/pdf/rfao_sept21.pdf
[21]http://www.hc-sc.gc.ca/food-aliment/english/organization/microbial_hazards/guidelines_for_raw_ground_beef.html

Guidelines for the Hygienic Production of Uncooked Fermented Comminuted Meat Products based on generic *E. coli* so as to include all STEC serotypes.[22]

These issues will need to be prioritized by the Committee and explicit questions for the risk assessors subsequently formulated should the Committee proceed with a quantitative risk assessment approach for the evaluation of these measures.

5. Available Information

A number of countries have evaluated the risk associated with foodborne EHEC (Table 4). Specifically, Canada has analyzed the risk associated with *E. coli* O157:H7 infection from consuming ground beef hamburgers (Cassin et al. 1998), sprouts (personal communication with Health Canada, January 2002), and juices (personal communication with Health Canada, January 2002), each of which have contributed to outbreaks or sporadic incidents of illness in Canada. An academic group in Canada has also assessed risk factors associated with on-farm *E. coli* O157:H7 prevalence in cattle (Jordan et al. 1999a, 1999b). The Netherlands chose to investigate steak tartare as the vehicle of transmission in their risk assessment because: (1) a steak tartare is thicker than a hamburger, therefore the risk of insufficient heating of the center is larger, (2) people tend to accept a partially raw tartare but do not accept a partially raw hamburger, and (3) tartare is sometimes consumed raw (e.g., as a tartare roll in snack bars). The U.S. has developed a farm-to-table risk assessment for *E. coli* O157:H7 in ground beef, in addition to a comparative risk assessment for *E. coli* O157:H7 in tenderized and non-tenderized steaks. Due to the smaller role of *E. coli* O157 serotypes in human illness in Australia, Australia has developed one risk assessment for O157:H7 STEC and another for all STEC in ground beef production and in fermented meat.

- FAO/WHO may find many of these risk assessments useful in the development of a risk assessment for Codex. Further evaluation of each is necessary.

Table 4: Risk assessments for *E. coli* O157:H7

Nation	**Topic**	**Reference**
Australia	Ground Beef[1]	Lammerding et al. 1999
Australia	STEC in Ground Beef[1]	Lammerding et al. 1999
Canada	Ground Beef Hamburgers	Cassin et al. 1998
Canada	Seeds/Beans and Sprouted Seeds/Beans[2,3]	Personal Communication with Health Canada
Canada	Unpasteurised Fruit Juice/Cider[4]	Personal Communication with Health Canada
Canada	Pre-harvest Husbandry Practices	Jordan et al 1999a, 1999b
Ireland	Beef/Beef Products	www.science.ulst.ac.uk/food/E_coli_Risk_ Assess.htm
Netherlands	Raw Fermented Products	www.research.teagasc.ie/vteceurope/S+Gprog /hoornstrasg.html
Netherlands	Steak Tartare	RIVM report 257851003/2001
U.S.	Ground Beef[5]	www.fsis.usda.gov/OPPDE/rdad/FRPubs/00-023NReport.pdf
U.S.	Tenderized vs. Non-tenderized Beef Steaks	Personal Communication with USDA

22 http://www.anzfa.gov.au/foodstandards/oldfoodstandardscodecontents/partcmeatcannedmeatandproductsthereof/c1meatgamemeatandrel686.cfm

[1]ANZFA Food Standard Code 1.6.1 sets Microbiological limits for total generic E. coli in a variety of foods. http://www.anzfa.gov.au/foodstandardscodecontents/standard16/index.cfm. Additionally, dairy products must be produced from pasteurized milk.

[2]Subsequent policy and management documents include "Consultation/Policy Document: A Dialogue on Developing a Risk Management Strategy for Sprouted Seeds and Beans".

[3]Subsequent policy and management documents include "Code of Practice for the Hygienic Production of Sprouted Seeds"

[4]Subsequent policy and management documents include "Code of Practice for the Production and Distribution of Unpasteurised Apple and Other Fruit Juice/Cider in Canada"

[5]The U.S. has a microbiological criterion that requires the absence of *E. coli* O157:H7 in raw ground beef

6. Data Gaps

Several data gaps have been identified based on currently available risk assessments for EHEC, including:

- Information describing the human health impact of EHEC in less developed nations.
- Commodities likely to be associated with EHEC foodborne illness in less developed nations.
- Data regarding the exposure dose of EHEC likely to cause illness in susceptible populations.
- Frequency and severity of illness among children ages 0 to 5 from EHEC, particularly among those that become ill from consuming ground beef and raw produce.
- Industry and consumer practices for various methods of manufacturing, cooking, and consuming ground beef and raw vegetables.
- Survival of EHEC on produce as a result of contamination by water or organic fertilizer.
- Information describing the critical contamination levels of meat products that may lead to cross contamination of uncooked produce.
- Information on the percentage of fresh leafy vegetables contaminated by bovine faeces containing EHEC
- Information on the maximum density of EHEC in ground beef and raw vegetable servings as related to matrix effects, competitive microflora, and environmental conditions (e.g., pH, water activity).
- Predictive microbiological data on the increase and decrease in the number of EHEC in ground beef and on raw vegetables under various storage and preparation conditions along with frequencies of occurrence of these storage and preparation conditions.
- Data on cross-contamination of EHEC between carcasses during carcass splitting.
- Time-temperature data (quantitative) for chillers in slaughter establishments.
- Marketing data on the proportion of beef ground at slaughter versus at retail.
- Data on retail and consumer storage, cooking, and consumption (frequency and serving size) patterns by type of ground beef meal (e.g., grilled hamburger in July and baked meat loaf in October).
- Descriptive epidemiologic information about sporadic cases of EHEC infection, including the month of disease onset, age, sex, hospitalizations, summary of clinical manifestations (including severe disease manifestations), and food vehicles involved (if known).
- Additional case-control studies of sporadic EHEC cases to calculate etiologic fraction attributable to ground beef.
- Information on processing and mitigation strategies to reduce the number of EHEC in meat and vegetables.

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