A surveillance study of *E. coli* O157:H7 and Enterobacteriaceae in Irish retail minced beef and beef burgers

Background

In 1999, the Food Safety Authority of Ireland (FSAI) issued a report entitled 'The Prevention of *E. coli* O157:H7 Infection: a Shared Responsibility' (www.fsai.ie). The report was produced by the Scientific Committee of the FSAI and contained many risk management recommendations. One recommendation in Chapter 8 on public health management was for the routine surveillance of *E. coli* O157:H7 in animals and foods. Acting on this recommendation, the FSAI commissioned Teagasc – The National Food Centre to carry out a 12-month study into the incidence of *E. coli* O157:H7 in minced beef and beef burgers on retail sale. The study commenced in March 2001 and ended in April 2002. This report is a summary of this study which will be reported in greater detail in the scientific literature.

Introduction

Escherichia coli (*E. coli*) is a common organism found in the intestinal tract of humans and animals. Most *E. coli* do not cause disease in humans, but certain types may cause diarrhoeal disease or more serious forms of illness. Among these, *E. coli* that may cause serious disease and death are a group called Verocytotoxigenic *E. coli* (VTEC). This group also includes types sometimes described as enterohaemorrhagic *E. coli* (EHEC). VTEC strains can be divided into many sub-groups, referred to as serogroups, based on variations in the structure of the surface of the bacterium. The principal serogroups are defined by chemicals on the surface of bacterial cells known as O-antigens and then subdivided into serotypes on the basis of other structural variations, e.g. H-antigens. The main serotype that frequently produces verocytotoxin and causes the classical illness associated with these pathogens is *E. coli* serotype O157:H7. Other serogroups, such as *E. coli* O26 and *E. coli* O45, are also included among VTEC as they have been shown to be capable of producing verocytotoxins.

The number of *E. coli* O157:H7 required to cause illness is very low. The precise infectious dose is not known but it has been reported to be between 10 and 100 cells. *E. coli* O157:H7 can survive the high acidity of the stomach and with such small numbers, infection can occur without any growth of the bacteria in food.

Infection with *E. coli* O157:H7 presents a wide range of clinical symptoms, including:

- non-bloody diarrhoea
- haemorrhagic colitis (bloody diarrhoea)
- haemolytic uraemic syndrome (HUS)
- thrombotic thrombocytopenic purpura (TTP).

Human infection with *E. coli* O157:H7 has been increasing since the early 1980's and has been reported from over 30 countries on six continents. Since 1996, the number of reported cases of *E. coli* O157:H7 in Ireland has steadily increased from 8 reported cases in 1996 to 76 cases in 1998, but has decreased slightly in recent years to 41 reported cases in 2000 and 52 in 2001. However, the true incidence of *E. coli* O157:H7 infections may exceed the reported number of cases, for a number of

reasons. Some infected persons are asymptomatic or do not seek medical care, some persons who obtain medical care do not provide a stool specimen and only some laboratories culture all stool samples for VTEC strains.

Livestock are the most important reservoir for most *E. coli* O157:H7, with cattle (both dairy and beef cattle) being the principal source. Internationally it is recognised that faecal shedding in cattle is intermittent, with maximum shedding rates being observed in the spring and summer months. Recent studies in the UK (Anon, 2001) have shown a herd prevalence of 23% in Scotland and 44% in England and Wales with an individual animal prevalence of 8% in Scotland and 4.7% in England and Wales. There is limited information on the prevalence of *E. coli* O157:H7 in the animal population in Ireland.

The source of contamination of foods is usually animal faecal material transferred into milk at milking, onto vegetables on manuring or onto carcases at carcase dressing. There are no nationally collated data on the prevalence of *E. coli* O157:H7 in Irish food and there is a limited number of laboratories that are equipped to test for *E. coli* O157:H7 in foods. In a small scale survey of meat samples purchased in Dublin butcher shops, carried out by Teagasc -The National Food Centre in 1998, 3.6% of salami samples were found to be contaminated with *E. coli* O157:H7. Epidemiological data from other countries indicate that the prime risk foods are undercooked beef burgers, unpasteurised milk, unpasteurised cheeses and raw vegetables. Besides animal manure, *E. coli* O157:H7 has been detected in soil and water. Waterborne outbreaks have occurred and both drinking water and recreational water have been implicated. Infection has been acquired through bathing in contaminated lakes and paddling pools.

Person to person spread can occur particularly in such places as institutions and child-care centres. Members of the same household may infect one another. Outbreaks due to direct contact with animals during farm visits have also been reported.

Occupationally acquired *E. coli* O157:H7 infection is a risk for farmers, abattoir workers and laboratory staff. There have been reports of laboratory staff becoming infected with *E. coli* O157:H7 while working with the organisms. In order to protect laboratory personnel against risks to their health and safety, *E. coli* O157:H7 has been classified under the EU Biological Agents Directive 97/59/EC as a Hazard Group 3 microorganism requiring Containment Level 3 laboratory facilities.

Definitive typing of *E. coli* O157:H7 is required to fully understand the epidemiology of this pathogen. Phenotypic and genotypic techniques now exist to facilitate tracing the organism from the food back to the original source. There is no database of definitively typed isolates from animals, food and humans in Ireland. However, Cherry Orchard Public Health Laboratory is undertaking definitive typing of strains from humans.

E. coli O157:H7 has no unusual resistance to heat and thorough cooking of foods (70^{o}C for 2 minutes or equivalent) will kill the organism. Pasteurisation of milk also effectively eliminates *E. coli* O157:H7. The minimum temperature for *E. coli* O157:H7 growth is 7^{o}C and the highest is 44.5^{o}C, with an optimum of 37^{o}C. *E. coli*

O157:H7 will grow well at room temperatures. The organism survives at low temperatures and resists freezing.

E. coli O157:H7 is a facultative anaerobe, that is, it can grow in environments where oxygen is present in low concentrations or absent. Therefore, modified-atmosphere packaging, which is commonly used to prevent spoilage in packaging fresh foods, has little effect on the growth or survival of the organism.

Unlike many foodborne disease-causing organisms, *E. coli* O157:H7 is tolerant of acidic environments and can survive up to two months at a temperature of 4^{o}C at pH 4.5. Outbreaks have been associated with consumption of acidic foods, such as dry raw meat salami, unpasteurised apple juice and mayonnaise. The acid resistance characteristic of *E. coli* O157:H7 provides it with the ability, at low infectious doses, to survive the acidity of the stomach and to invade the gut causing disease.

E. coli O157:H7 can remain viable in soils, water and manure for considerable periods and the organism has been shown to survive for several months in manure and contaminated grassland.

Brief Methodology

Samples of loose and pre-packed minced beef and beef burgers were collected from retail outlets in the Republic of Ireland over a 12-month period. Quarterly samples were taken in each of the 26 counties, targeting 2 large towns (2 shops each) and one small town (1 shop) in each county. In each county, the five premises consisted of 3 supermarkets and 2 butcher shops. Hence, each shop was sampled 4 times in the course of the study.

Sampling was conducted over four periods:

- 25th **March** 2001 to 21st **June** 2001 (inc samples from 18th and 19th April 2002 in Louth due to Foot and Mouth restrictions in 2001)
- 28th **June** 2001 to 7th **September** 2001
- 11th **October** 2001 to 18th **January** 2002
- 24th **January** 2002 to 12th **April** 2002

All samples were analysed for *E .coli* O157:H7 by method ISO 16654 and Enterobacteriacea by British Standard method BS 5763. *E. coli* O157:H7 were also enumerated by direct plate count on CT-SMAC using latex agglutination as a confirmatory test. Examination of virulence factors was carried out on all *E. coli* O157:H7 isolates.

Key Results

- **43** samples from 1,533 samples (2.8%) tested positive for *E. coli* O157:H7.
    - 22 of the 43 samples were positive by enrichment only (i.e. < 3 cfu/g)
    - 21 of the samples carried *E. coli* O157:H7 at levels between $\log_{10}$ 0.51-4.03 cfu/g
    - 32 of the 43 samples originated in supermarkets, with the remaining 11 samples originating in butcher shops

        - Supermarkets were sampled more frequently than butcher shops and therefore there was no significant difference in the prevalence of *E. coli* O157:H7 in samples taken from the two shop types.
    - There was no significant difference at the 95% confidence level between the prevalence of *E. coli* O157:H7 in any of the five product types tested.
    - all positive sample isolates contained virulence factors
        - 41 contained both verotoxin genes plus the attachment and effacement genes
        - 2 contained only one of the verotoxin genes and the effacement gene
    - Clusters of positive samples were sometimes found at the same sampling time in certain towns.
        - A minority of clustered positive samples that were taken in the same supermarket chain may have originated from a common supply chain. However, for the majority of clustered positive samples no such supply link could be made in the absence of batch identification codes.

- Examination of the prevalence data from this study by stochastic methods allowed for an estimation of the true contamination rate for *E. coli* O157:H7 in minced beef and beef burgers in Ireland. Taking into account the uncertainty in the estimate of prevalence inherent in the sampling methodology, we are confident, at the 95% level, that the true prevalence of *E. coli* O157:H7 in retail samples of Irish minced beef and beef burgers is less than or equal to 3.6%.

- Seasonality in the positive samples was observed
    - 25th March 2001 to 21st June 2001 (3.23% positive)[∝]
    - 8th June 2001 to 7th September 2001 (2.35% positive)
    - 11th October 2001 to 18th January 2002 (1.03% positive)
    - 24th January 2002 to 12th April 2002 (4.78% positive)

- Different product types yielded different prevalence of *E. coli* O157:H7
    - 2.8% (13 of 457) of fresh packaged minced beef (max product prevalence found in the study)
    - 2.1% (3 of 140) fresh unpackaged burger from butchers shops (min product prevalence found in the study)

- There was no correlation between the presence of *E. coli* O157:H7 and the numbers of Enterobacteriaceae in the samples

- There was no significant difference between the Enterobacteriaceae counts on minced beef or beef burgers and the type of shop selling the product (e.g. supermarket vs. butcher shops)

[∝] included samples from 18th and 19th April 2002 in Louth due to Foot and Mouth restrictions in 2001

- Enterobacteriaceae counts were significantly higher on fresh unpackaged minced beef than on fresh unpackaged beef burgers irrespective of retail premises.

Discussion

The study clearly shows that *E. coli* O157:H7 is present in Irish minced beef and beef burgers at unacceptable and potentially hazardous levels. Table 1 shows the results of similar surveys in other countries. Whilst not all methodologies were comparable in these studies, it is clear that the present findings are comparable to those of other studies in countries such as Canada (1990), USA (1987, 1991) and Spain (1996).

A previous survey carried out in Ireland (Walsh *et al*, 1997 listed in Table 1) found a much lower prevalence of *E. coli* O157:H7 in retail and wholesale minced beef. This finding of a prevalence of 0.2% is significantly different at the 99% confidence level from the 2.8% prevalence reported here. Although the 1997 study only took samples in the Munster region of Ireland over a 12 month period it is unlikely that the magnitude of this difference could be accounted for by this regional sampling difference given the national nature of the beef supply chain. This comparison would suggest that the prevalence of *E. coli* O157:H7 in minced beef has increased over the past four years.

Table 1 (adapted from De Boer and Hauvelink, 2001) Prevalence of *E. coli* O157:H7 in beef products compared by country (This study in grey)

Product	*Country*	*No positive/ No analysed (%)*	*Year*
Ground Beef	USA	6/164(3.7)	1987
Ground Beef	USA	3/107(2.8)	1991
Ground Beef	Ireland	1/568(0.2)	1997
Ground Beef	Norway	0/1319(0)	1997
Ground Beef	Canada	4/165(2.4)	1990
Ground Beef	Egypt	3/50(6.0)	1996
Ground Beef	The Netherlands	6/571(1.1)	1999
Ground Beef	Spain	3/58(5.2)	1996
Ground Beef	USA	1/76(0.3)	1992
Ground Beef	Italy	9/564(1.6)	1998
Ground Beef	USA	0/563(0)	1996
Beef Burgers	UK	3/1015 (0.3)	1998
*Beef Burgers**	*Ireland*	*27/967 (2.8)*	*2002*
*Ground Beef**	*Ireland*	*16/566(2.8)*	*2002*

* this reported study

A further important finding in this study is the numbers of *E. coli* O157:H7 that can be associated with a particular product. Table 2 and Figures 1 and 2 show the pattern of numbers of *E. coli* O157:H7 in beef burgers and minced beef.

Table 2 Enumeration of *E. coli* O157:H7

Product	**No of samples positive for *E. coli* O157:H7**	**No of positive samples with *E. coli* O157:H7 below detection limit (3 cfu/g)**	**No of positive samples with *E. coli* O157:H7 above the detection limit (count range log_{10} cfu/g)**
Minced Beef	27	11 (41%)	0.81 - 4.03
Beef Burger	16	11 (69%)	0.51 – 3.04

Figure 1

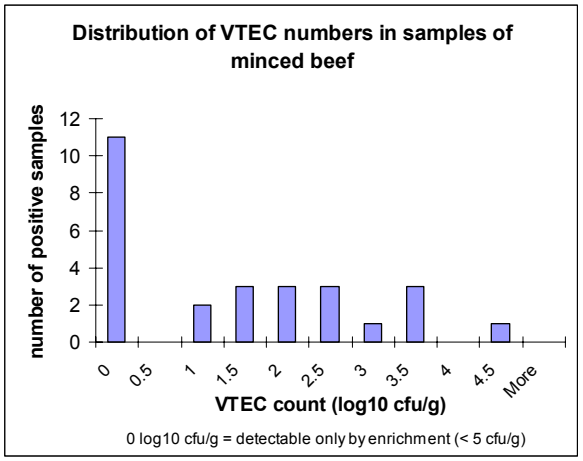
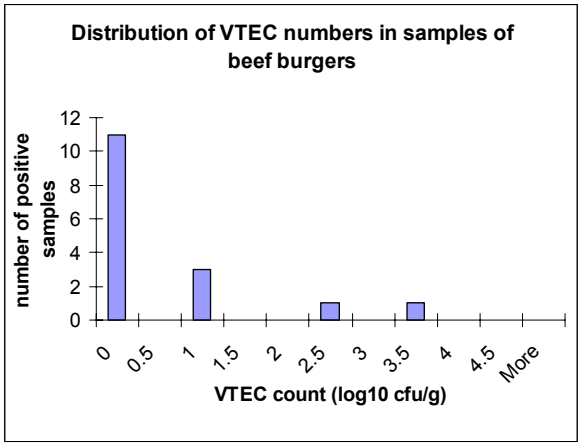


Figure 2



A population of 10,000 *E. coli* O157:H7 per gram in minced beef (log_{10} 4.0 cfu/g) could increase the risk of the organism surviving cooking and could also increase the risk of cross contamination if preparation practices in the home or retail outlet were not totally hygienic.

E. coli O157:H7 has an estimated infectious dose of 10 organisms. Therefore, a combination of relatively high prevalence and occasional high contamination levels presents a potential risk to public health. However analysis of human infection data from the National Disease Surveillance Centre showed no correlation between the geographical incidences of *E. coli* O157:H7 positive samples of minced beef and beef burger found in the present study and the reported incidences of *E. coli* O157:H7 infection in the same geographical area over the same time period. Therefore we must assume that either cases of foodborne disease are unreported, which is unlikely or that the final cooking and handling of the products by the consumer is preventing live organisms from being ingested. This puts an unnecessary burden on the final control point in the food chain. Clearly further action is required within farms and abattoirs to reduce the prevalence and numbers of *E. coli* O157:H7 in beef.

This study showed that the prevalence of *E. coli* O157:H7 in minced beef and beef burgers at retail level was highest in the first six months of the year. This is slightly different to the generally accepted model that faecal shedding of *E. coli* O157:H7 in cattle increases in spring and summer (Blanco *et al,* 2001). However this aspect would require further research before attributing any cause and effect relationship.

Finally, for *E. coli* O157:H7 the survey found no correlation between this organism and Enterobacteriaceae counts. *E. coli* O157:H7 was recovered from samples with both low and high Enterobacteriaceae counts. These findings are in line with other studies that show that Enterobacteriaceae cannot be used as indicator organisms for the presence of *E. coli* O157:H7. Interestingly, there was no significant difference between the Enterobacteriaceae counts on samples from butcher shops and supermarkets which indicates that hygienic practices, whilst different, are equally effective within their respective retail setting. This had no bearing upon the prevalence of *E. coli* O157:H7 in samples from the two types of establishment.

The fact that *E. coli* O157:H7 prevalence in minced beef and beef burgers was not significantly different irrespective of retail outlet, packaging format and mincing environment (industrial vs. shop produced) suggests that the prevalence is unaffected by the retail distribution chain and may be solely a function of the contamination of meat used in the production of the minced beef. This in turn suggests that apart from maintaining temperature control and hygienic handling practices there is little more that retailers can do to reduce the prevalence of *E. coli* O157:H7 in the products they sell, provided that they prevent cross contamination at the mincing stage if this is carried out at the retail premises. The onus for better control must be on the producer and the slaughter facility.

Conclusions

The current prevalence of *E. coli* O157:H7 in minced beef and beef burgers coupled with the emergence of occasional gross contamination of comminuted beef products at retail level presents a real risk to public health. It is likely that this risk is only being controlled by the cooking process to which the meat is subjected in the consumers' home. In the case of samples with gross contamination, the risk of cross contamination in the home is increased.

In reality, the prevalence rates are so low that it is unlikely that an economically viable end product testing regime could control *E. coli* O157:H7 in minced beef and

beef burgers. This is not to say that such testing should not be done, however it should be undertaken with knowledge of the level of detection and would only ensure major contamination would not be missed. The emphasis has to be on controlling the organism on the farm and in the slaughter plant and cutting hall. This can be done to a certain extent by a combination of Good Agricultural Practice (GAP), Good Manufacturing Practice (GMP) and Hazard and Critical Control Point (HACCP)[βα] systems along with an increased awareness of the risks by food handlers. It is only when everything that can be done is being done that it is reasonable to expect consumers to eliminate any residual risk by cooking in the domestic environment. Much work has been done in this area in the last few years. However, the results of this study leads us to two possible conclusions.

EITHER

1. The recommended changes to animal husbandry practices, slaughterhouse GMP and the introduction of HACCP are not in themselves capable of reducing the prevalence of *E. coli* O157:H7 in minced beef and beef burgers to lower levels than 4%.

OR

2. The recommended changes to animal husbandry practices, slaughterhouse GMP and the introduction of HACCP are capable of reducing prevalence of *E. coli* O157:H7 in mince beef and beef burgers to below 4%, but are not being fully implemented.

The FSAI's1998 report on the control of *E. coli* O157:H7 emphasised 'a shared responsibility'. At present there is an unacceptable burden on the consumer to control the risk at a point in the food chain that is least likely to be controlled. Given the morbidity and mortality associated with *E. coli* O157:H7, if the present production regime in Ireland is unable to reduce the risk sufficiently then additional efforts must be made. For example, pre-cooked frozen beef burgers are already available in Ireland. Furthermore, irradiation is a proven process for eliminating pathogenic micro organisms in raw meat (Olsen, 1998). The provision of minced beef products that are rendered free from *E. coli* O157:H7 by these means should at least be considered for those catering for vulnerable groups of the population, such as the elderly and young infants.

Meanwhile, definitive typing of all isolates of *E. coli* O157:H7 from humans, animals and foods is essential to fully understand the sources and the routes of transmission of this pathogen. The current arrangements in Ireland do not adequately address this requirement.

[β] Commission Decision 471/2001/EC [L Journal 165/48], extends Hazard Analysis and Critical Control Points (HACCP) and microbiological checks to slaughter and cutting plants approved under Council Directives 64/433/EEC, as amended (FRESH Meat) and 71/118/EEC, as amended (Fresh Poultry Meat).

[α] FSAI Guidance Note No 8; 2002. The Implementation of Food Safety Management Systems in Beef and Lamb Slaughter Plants based on HACCP Principles.

References

Anon (2001) Chapter 5: Animals and environment: Understanding the hazard. P50-56 in The task force on E. coli O157: Final Report. Scottish Executive

Blanco. J, Blanco, M., Blanco, J.E., Alonso, A., Gonzalez, E. and Fernardez, M.I. (2001) Epidemiology of verocytotoxigenic *Escherichia coil* (VTEC) in ruminants. In, Verotoxigenic *E. coli* eds. Duffy, G., Garvey, P. and McDowell, D.A. Food Science and Nutrition Press, p120 (ISBN 0 917678 – 52 – 4)

De. Boer, E. and Heuvelink, A.E. (2001) Foods as vehicles of VTEC infection. In, Verotoxigenic *E. coli* eds. Duffy, G., Garvey, P. and McDowell, D.A. Food Science and Nutrition Press, p120 (ISBN 0 917678 – 52 – 4)

Olsen D.G. (1998) Irradiation of foods. Food technology. 52 (1) :56-62

Walsh, L., Dooge, D. and Hill, C. (1997) Screening of *Escherichia coli* O157:H7 in Irish ground beef using two commercial detection systems. Irish Veterinary Journal 50:111-115.