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Dr Lucy Rivas, Dr Rob Lake, Peter Cressey, Nicola King,
Dr Beverley Horn, Dr Brent Gilpin

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RISK PROFILE (UPDATE): SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI* IN RED MEAT AND MEAT PRODUCTS

Prepared for the Ministry for Primary Industries
under project MRP/11/01 - Microbiological Risk Profiles,
as part of an overall contract for scientific services

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Dr Stephen On
Chief Scientist
Environmental Science Group

Dr Rob Lake
Project Leader

Dr Muriel Dufour
Peer Reviewer

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This Risk Profile still uses the names NZFSA and MAF for documents produced during the existence of these organisations.

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GLOSSARY AND ABBREVIATIONS

a_w	Measure of water activity (max = 1.000 = pure distilled water)
ACMSF	Advisory Committee on Microbiological Safety of Foods
CAC	Codex Alimentarius Commission
CI	Confidence Interval
EHEC	Enterohaemorrhagic *E. coli*
EU	European Union
FSANZ	Food Standards Australia New Zealand
FSP	Food Safety Programme (under the *Food Act* 1981)
HACCP	Hazard Analysis Critical Control Point
HC	Haemorrhagic Colitis
HUS	Haemolytic Uraemic Syndrome
MAF	Ministry of Agriculture and Forestry (now part of MPI)
MPI	Ministry for Primary Industries
MPN	Most probable number
NZFSA	New Zealand Food Safety Authority (now part of MPI)
OR	Odds Ratio
PCR	Polymerase Chain Reaction
PFGE	Pulsed Field Gel Electrophoresis
pH	Measure of acidity (min = 0 = most acidic; max = 14)
RMP	Risk Management Programme (under the *Animal Products Act* 1999)
STEC	Shiga Toxin-producing *E. coli*
US	United States (of America), shortened version often officially used
USA	United States of America
USDA	United States Department of Agriculture
FSIS	Food Safety and Inspection Service (USDA)
VTEC	Verocytotoxigenic *E. coli* (synonym of STEC)

SUMMARY

This Risk Profile considers Shiga toxin-producing *Escherichia coli* (STEC) in red meat and meat products. This is an update of a Risk Profile published in 2002.

Infection by STEC in humans usually results in bloody diarrhoea (haemorrhagic colitis), and a small proportion of infected people will go on to suffer more serious outcomes including haemolytic uraemic syndrome (HUS), and in 1-4% of HUS patients death. While 70% of patients with HUS recover completely, the remainder suffer a range of long term sequelae primarily including chronic renal disease (proteinuria, hypertension, chronic kidney disease (CKD), end-stage kidney disease (ESKD)), and less frequently gastrointestinal complications, neurological disorders and diabetes mellitus.

This Risk Profile has been commissioned in order to address the following specific risk management question:

- Has the risk from STEC in red meat changed since the previous Risk Profile in 2002?

Since the previous Risk Profile, a number of studies (at both processing plants and retail sources) have shown that STEC can be detected (i.e. toxin genes *stx1* and/or *stx2* present in enrichment broth samples) in meat from cattle, sheep, and pigs in New Zealand, at up to 14.7% in one survey. However, the prevalence of STEC which contain virulence genes (*eaeA* and *hlyA*) in addition to the *stx* genes is much lower (<2%). The data collected by the most recent New Zealand survey indicate that STEC are present in red meats at very low concentrations, with a majority of counts of <0.33 MPN/g.

Since the previous Risk Profile, many studies have been undertaken to assess the carriage of STEC by red meat livestock in New Zealand. STEC have been shown to occur in cattle, dairy cows, sheep and lambs, with a higher prevalence found in very young calves. As with red meat, the prevalence of animal faecal or hide samples containing *stx1* and/or *stx2* genes is much higher than the prevalence of isolates containing additional virulence genes (*eaeA* and *hlyA*), or serotypes associated with human infections. No studies have been undertaken to examine the prevalence of STEC in live pigs or deer.

Red meats are commonly consumed products with approximately 69% of respondents aged 15 years or over in the 2009 Adult Nutrition Survey reporting consumption of red meat (beef, veal, sheep meat, pig meat and venison) during the 24 hours prior to the survey.

The normal acidity of meat and processed meat products and their storage under refrigeration or freezing suggests that levels of contamination are unlikely to increase significantly during storage, provided refrigeration temperatures (< 8°C) are maintained. STECs are readily inactivated by normal cooking temperatures.

STEC are normally present only on the surface of intact muscle meat, but may become internalised when meat is minced or injected. Most incidents of human infection overseas occur when STEC present in minced meat products such as sausages and hamburgers, are undercooked prior to consumption. A survey of New Zealand consumers found that no consumer preferred their sausages or minced beef/hamburgers cooked rare.

The annual rate of reported STEC infection rose from 2006 to 2009, was relatively stable from 2009 to 2012, and has increased again in 2013. The highest age-specific rates of infection continue to be observed in young children. Up to 43% of cases of STEC infection were hospitalised in the years 2006 to 2013, and one death was reported in 2009. *E. coli* O157:H7 continues to be the predominant serotype isolated from New Zealand cases of STEC infection. However, New Zealand clinical laboratories do not have consistent protocols and procedures in place for detecting non-O157 STEC and there are no national testing protocols for the isolation of non-O157 STEC.

The 2002 Risk Profile concluded "there is currently little information to suggest that transmission of STEC via red meat is occurring in New Zealand". This finding was based on information at the time that indicated a low prevalence of STEC on New Zealand meat and a lack of epidemiological evidence linking red meat consumption to cases of STEC infection in New Zealand.

Since the 2002 Risk Profile a number of studies have reported that STEC are widespread in both retail meat and livestock in New Zealand. However strains exhibiting additional virulence markers associated with human disease (*eaeA* and *hlyA*) are at a lower prevalence (less than 2%). Should exposure occur, the risk of infection from red meat would be high, as recent dose-response models predict that very low numbers of cells provide a high risk of infection.

Nevertheless, information from outbreaks since 2002 and a case-control study have not provided evidence of human STEC infection via red meat in New Zealand. Therefore the conclusion of the previous Risk Profile is unchanged. Cooking of red meat before consumption and good hygiene practices during food preparation are important barriers to exposure, and will mitigate the risk from red meat.

1 STATEMENT OF PURPOSE

This document updates the 2002 Risk Profile considering Shiga toxin-producing *Escherichia coli* (STEC) in red meat and meat products (Lake *et al.*, 2002).

This update is not a stand-alone document and refers to information presented in the 2002 document, which can be accessed from: http://www.foodsafety.govt.nz/elibrary/industry/Risk_Profile_Shiga-Science_Research.pdf.

The purpose of this update is to critically review new information to answer the following risk management question:

- Has the risk from STEC in red meat changed since the previous Risk Profile in 2002?

Risk Profiles provide scientific information relevant to a food/hazard combination for risk managers and describe potential risk management options (NZFSA, 2010).[1]

The literature on STEC in red meat is extensive. The focus of this update has been on studies that have been performed in New Zealand, and overseas studies that are informative about attribution and risk management interventions.

[1] Risk Profiles commissioned by MPI and its predecessors can be viewed at: http://www.foodsafety.govt.nz.

2 HAZARD AND FOOD

2.1 The Pathogen: Shiga Toxin-producing *E. coli* (STEC)

KEY FINDINGS

Serotyping remains an important STEC typing method, but since the 2002 Risk Profile there has been a greater emphasis on virulence genes as indicators of pathogenicity. The addition of six STEC serotypes having adulterant status in the United States has provided more data on non-O157 serotypes through research, food testing and public health surveillance.

A number of mainly plasmid borne virulence genes have been identified in STEC, with the presence of more virulence genes generally associated with more severe disease outcomes (Lynch *et al.*, 2012). The presence of virulence genes varies among isolates sharing the same serotype, with many investigators suggesting that detecting multiple genes better discriminates between STEC strains and helps determine the potential for an STEC isolates' ability to cause disease

Appendix 1 contains additional information on STEC.

2.1.1 Nomenclature

E. coli with the Shiga toxin 1 or 2 gene (*stx*$_1$ or *stx*$_2$) are classified as Shiga toxin-producing *E. coli* (STEC) or verocytotoxin-producing *E. coli* (VTEC). Enterohaemorrhagic *E. coli* (EHEC) are the subset of STEC that are capable of causing haemorrhagic colitis (HC) and haemolytic uraemic syndrome (HUS). The system of classifying *E. coli* by pathotype (Nataro and Kaper, 1998) has recently been challenged by a foodborne outbreak in Germany during 2011, caused by an enteroaggregative *E. coli* (EAEC) strain (*E. coli* O104:H4) that had acquired the ability to produce Shiga toxin (i.e. had become an STEC) yet did not have any of the other virulence markers typically associated with EHEC (Beutin and Martin, 2012; Clements *et al.*, 2012). *E. coli* O104:H4, while it is a hybrid of EHEC and EAEC, is still considered as an STEC (Croxen *et al.*, 2013). The ability of bacteria to acquire (or lose) genetic material makes it difficult to be definitive about the classification of pathogenic *E. coli*.

2.1.2 Pathogenicity

While more than 100 serotypes of STEC have been associated with human disease, *E. coli* O157 causes 50 to 90% of cases, with most of the remaining cases caused by just six serotypes: O26, O45, O103, O111, O121 and O145 (Scallan *et al.*, 2011).[2] Many STEC serotypes have been isolated that have not been associated with human disease, although this does not mean that they are not capable of causing illness.

A number of mainly plasmid borne virulence genes have been identified in STEC, with the presence of more virulence genes generally associated with more severe disease outcomes (Lynch *et al.*, 2012). The presence of virulence genes varies among isolates sharing the same serotype, with many investigators suggesting that detecting multiple genes better discriminates between STEC strains and helps determine the potential for an STEC isolates' ability to cause disease (Brandt *et al.*, 2011). Identified virulence genes include:

[2] A colloquial term for O26, O45, O103, O111, O121 and O145 is the "super-six". The term "super-seven" refers to these serotypes plus O157.

- The *stx1* and *stx2* genes that encode for Shiga toxins that inhibit protein synthesis causing cell death. There are now at least three recognised subtypes of the toxin Stx1 (Stx1a, Stx1c and Stx1d) and seven subtypes of Stx2 (Stx2a, Stx2b, Stx2c, Stx2d, Stx2e, Stx2f and Stx2g) (Baylis, 2009).
- The *eae*A gene which encodes for intimin, an outer membrane surface adhesion responsible for attachment of STEC to intestinal cells (Croxen *et al*., 2014).
- The *hylA* or *ehxA* genes which encode for enterohaemolysins that enhance the effect of Shiga toxins (Croxen *et al*., 2014).
- The *cdtABC* cluster of genes which encode for cytolethal distending toxin that damages host DNA (Croxen *et al*., 2014).
- The *aaiC* (secreted protein of EAEC) and *aggR* (plasmid-encoded regulator), both associated with increased virulence (EFSA, 2013).

The documents reviewed for this update usually only targeted the *stx1* and *stx2* genes and occasionally *eaeA* and *hlyA/ehxA*.

A categorisation scheme that classifies STEC serotypes on the basis of human disease incidence and severity (Karmali *et al.*, 2003) was recently reviewed by the European Food Safety Authority (EFSA) Panel on Biological Hazards over concerns that testing regimes were too focussed on serotypes classified as 'high risk' by this scheme, thus failing to recognise the potential for other serotypes to cause human disease (EFSA, 2013). The Panel concluded that "there is no single or combination of marker(s) that defines a "pathogenic" VTEC." However, the Panel recognised that STEC strains positive for *stx2* and either *eae* or the combination of *aaiC* and *aggR* were associated with a higher risk of more severe illness than other virulence gene combinations. Both of these approaches use a combination of serotyping and detection of virulence genes to predict human health risk (see (EFSA, 2013) for details), but EFSA acknowledged that the human health risk of STEC isolates that do not possess the target genes or serotypes cannot be inferred.

2.2 The Food: Red Meat and Red Meat Products

KEY FINDINGS

Pork production is similar to the 2002 Risk Profile, but production of other meats has decreased since that year. Nevertheless, the New Zealand meat industry still represents a major part of the primary production sector by value, and most production (>80% of lamb, mutton, beef and venison) is exported. The exception is pork, for which 44% of the supply was imported in 2013.

2.2.1 Definitions

For the purpose of this Risk Profile 'red meat' is taken to include the skeletal muscular tissue and associated materials (fat and other tissues) from the main commercial meat species i.e. cattle, sheep, pigs and deer. This Risk Profile also addresses veal, which is the meat of very young cattle (calves).

2.2.2 Product characteristics

Meat contains a high proportion of water and protein. All fresh red meats have internal water activities (a_w) of >0.99 which provides a suitable environment for microbial growth (ICMSF, 2005). STEC can be found on the surfaces of carcasses where water activity will be lower due to moisture loss (unless wrapped in plastic).

2.2.3 The food supply in New Zealand: Red meat and red meat products

2.2.3.1 The red meat industry

The Meat Industry Association of New Zealand (MIA) is a voluntary trade association representing New Zealand meat processors, marketers and exporters. MIA member companies operate approximately 60 processing plants throughout the country, representing companies supplying the majority of New Zealand sheep and beef meat exports. Sheep meat and beef exports make up 13% of New Zealand's exports by value (22% of New Zealand's primary sector revenue).[3] For the year ending June 2012, the meat industry earned export revenue of $6.1 billion.[4]

2.2.3.2 Production

Pork production is similar to statistics presented in the 2002 Risk Profile, but production of other meats has decreased since that year. Nevertheless, the New Zealand meat industry still represents a major part of the primary production sector by value.

Livestock slaughter and export statistics for the year ending 30 September 2013 are shown in Table 1.

Table 1: Livestock numbers, production and export for New Zealand, year ending 30 September 2013

Livestock type	Total inspected slaughter at export plants and abattoirs (million head)	Meat production, bone in basis (000 tonnes)	Meat exports (000 tonnes)
Lamb	21.3	382.4	313.1
Sheep	4.3	105.6 (mutton)	85.0
Cattle (including calves)	4.3	628.3 (beef and veal)	366.5 (beef and veal)
Pigs	0.7	47.1	0.1
Deer	0.4	22.9	13.2

Source: Compendium of New Zealand Farm Facts. http://www.beeflambnz.com/Documents/Information/Compendium%20of%20New%20Zealand%20farm%20facts.pdf accessed 23 October 2014

[3] http://www.mia.co.nz/about_us/ accessed 10 July 2013

[4] http://www.mia.co.nz/docs/statistics/2012/Summary%20of%20red%20meat%20industry%20exports,%20year%20ended%20June%202012.pdf accessed 10 July 2013

2.2.3.3 New Zealand exports

New Zealand is a major exporter of beef and sheep meat, with approximately 832,000 tonnes of meat (including 66,942 tonnes of offals) exported in the 12 months to 30 September 2013.[5]

New Zealand exports only a small, but increasing amount of pork (approximately 115 tonnes in 2012), mainly to Pacific Island nations and Hong Kong.[6]

In 2013, 13,237 tonnes of venison were exported.[7]

2.2.3.4 New Zealand imports

New Zealand imports relatively small amounts of beef and sheep meat, according to data from Statistics New Zealand.[8] For the 2012 year approximately 3,500 tonnes of beef and 1,900 tonnes of sheep meat were imported from Australia; these values are slightly lower than those reported in the 2002 Risk Profile.

Imports of pork have increased since the 2002 Risk Profile. In the year to April 2013 the pork supply in New Zealand included 45,117 tonnes of imported pork (44% of total supply).[9] Imports came from Canada (22%), Scandinavia (32%), Australia (14%) and the USA (23%). Most (96%) of imported pork was in the form of frozen cuts.

Processed meats were also imported, principally from Australia. In 2012, total imports of meat preparations were approximately 4,000 tonnes.

2.3 Behaviour of STEC in Red Meat

KEY FINDINGS

The normal acidity of meat and processed meat products and their storage under refrigeration or freezing suggest that levels of contamination are unlikely to increase significantly during storage, provided refrigeration temperatures (< 8°C) are maintained. The organism is rapidly inactivated at temperatures above 60°C, with D times at these temperatures being generally less than 5 minutes.

A proportion of red meat livestock carry STEC in the intestinal tract at slaughter. The pathogen may also be present on the hide and hooves. Care taken during evisceration and hide removal can limit, but not entirely prevent, contamination of the carcass.

[5] http://www.beeflambnz.com/Documents/Information/Compendium%20of%20New%20Zealand%20farm%20facts.pdf accessed 23 October 2014
[6] http://www.stats.govt.nz/infoshare/TradeVariables.aspx?DataType=TEX accessed 27 June 2013
[7] http://www.deernz.org/about-deer-industry/nz-deer-industry/deer-industry-statistics/glance-industry-statistics accessed 15 October 2014
[8] http://www.stats.govt.nz/infoshare/TradeVariables.aspx?DataType=TIM accessed 27 June 2013
[9] http://www.nzpork.co.nz/Publications/ImportsReport.aspx accessed 20 June 2013

2.3.1 Survival on red meat

E. coli O157:H7 survives on red meat under both refrigeration and frozen storage, although reductions of up to 2 $\log_{10}$ in numbers have been observed over time under these conditions. Examples of published research include:

- *E. coli* O157:H7 numbers remained constant for 14 days on aerobically or vacuum packed 1 cm thick beef steaks stored at 4°C, and presence of background microflora made no difference (Berry and Koohmaraie, 2001).

- In burger meat refrigerated at 2°C for 4 weeks *E. coli* O157:H7 numbers declined by 1.9 $\log_{10}$ CFU/g (Ansay *et al.*, 1999).

- *E. coli* O157:H7 has been shown to survive when inoculated into ground beef, made into patties, and stored frozen at -20°C for 1 year, with only a 1-2 $\log_{10}$ CFU/g reduction in concentration observed (Ansay *et al.*, 1999). More recently a reduction of approximately 1 $\log_{10}$ CFU/g *E. coli* O157:H7 on frozen ground beef after 60 days storage at -20°C has been reported, with a further reduction of another 1 $\log_{10}$ CFU/g after a total of 90 days (Keeling *et al.*, 2009). An investigation of freezing profiles concluded that greater decreases in *E. coli* O157:H7 numbers might be achieved by extending the length of the freezing plateau at around 0 to -2°C, and by pre-chilling to 12°C prior to freezing (Dykes, 2006).

- In trials where beef patties were first frozen (-20°C for 24 hours) and then thawed (refrigerated, ambient temperature, microwaved) *E. coli* O157:H7 reduced by between 0.62 and 2.52 $\log_{10}$ CFU/g, depending on the strain (Sage and Ingham, 1998). No defrosting method was consistently superior for reducing pathogen numbers.

A cooking time of 2 minutes at 70°C will deliver a 7 log (D) kill (Stringer *et al.*, 2000).

In *sous vide* ground beef the D time of a cocktail of strains of *E. coli* O157:H7 was shown to be 67.7 minutes at 55°C, 2.0 minutes at 62.5°C, and 1.89 minutes at 65.6°C (Juneja *et al.*, 2009; Keeling *et al.*, 2009). In work where inoculated ground beef had been stored refrigerated or frozen prior to testing, the D times ranged from 0.3 to 6.3 minutes at 62.8°C (Zhao *et al.*, 2004) depending on the specific *E. coli* O157 isolate studied and conditions used. Z values of 6°C and 7.6°C in minced beef (Huang and Juneja, 2003; Juneja *et al.*, 1997), close to 5°C in lean and fatty minced beef (Line *et al.*, 1991) and 4.8°C (Ahmed *et al.*, 1995) in lean beef have been reported.

The sensitivity of *E. coli* O157:H7 to heat inactivation can be affected by pre-freezing and quality of the burger meat (Byrne *et al.*, 2002), the pH of the meat, and acidulant used (Juneja and Novak, 2003). Increased heat sensitivity can also be achieved by the addition of a variety of materials, for example tea leaf and apple skin powders (Juneja *et al.*, 2009), acidic calcium sulphate and lactic acid combined (Zhao *et al.*, 2004), lactic acid (Mukherjee *et al.*, 2009), and carvacrol and cinnamaldehyde (Juneja and Friedman, 2008).

2.3.2 Contamination of red meat

Primary processing of livestock for red meat involves (ICMSF, 2005):

- Stunning; (e.g. electrical / captive bolt / carbon dioxide gas);
- Sticking (kill) usually by cutting the carotid arteries;
- Bleeding;
- Skinning (or scalding and dehairing for pigs/goats);
- Evisceration (inspection of offal and corresponding carcass and head, edible offals separated from other offal in a separate area of the abattoir);
- Trimming and washing;
- Air cooling of carcasses; and,
- Grading/cutting and packaging.

STEC are carried asymptomatically within the gastrointestinal tract of livestock, and studies in cattle indicate colonisation of the rectoanal junction plays an important role in faecal carriage (Cobbold *et al.*, 2007; Kaspar *et al.*, 2010). A proportion of red meat livestock carry STEC at slaughter either (i) in the intestinal tract or (ii) on the hide or hooves which have been contaminated by faecal matter.

STEC on the carcass can be transferred to meat cuts as the animal is further processed, and can also be transferred between animals via meat processing equipment (ICMSF, 2005). STEC contamination of primal meat cuts will be surface contamination only. When primal cuts are further processed (e.g. minced/ground meat, sausages), microorganisms are homogenised throughout the product.

Care taken during evisceration and hide removal can limit, but not entirely prevent, contamination of the carcass (ICMSF, 2005) (Ogden *et al.*, 2002; Pearce *et al.*, 2004). Data supporting a positive association between the concentration of *E. coli* O157:H7 in the faeces and the likelihood of the carcass being contaminated has been presented (Fegan *et al.*, 2005).

Cattle carriage of *E. coli* O157:H7 is dynamic with occasional high prevalence and periods of apparent absence (Pennington, 2010). STEC can survive in open and water environments, with wild and farm animal species acting as reservoirs (Chekabab *et al.*, 2013), leading to the continual risk of herds becoming re-infected. A study including herds from 30 farms in England and Wales found an increased risk of a herd being positive for *E. coli* O157 when using indoor housing (OR 4.9 95% CI: 1.15-20.8) (Ellis-Iversen *et al.*, 2009). Feed has been identified as both a possible source of STEC infection and also a factor in the prevalence and shedding of STEC in cattle (Jacob *et al.*, 2009; Soon *et al.*, 2011). A review provides evidence that STEC can survive for extended periods (weeks/months) in faeces, soil and water (Fremaux *et al.*, 2008).

The concentration shed in faeces can exceed 5 $\log_{10}$ CFU/g (Omisakin *et al.*, 2003). While "super shedders" can shed up to 7 $\log_{10}$ CFU/g of *E. coli* O157:H7, most faecal samples that are positive for the bacterium contain <2 $\log_{10}$ CFU/g (Chase-Topping *et al.*, 2007). The presence of a high level shedder of *E. coli* O157:H7 on a farm was found to be associated with a high proportion of low level shedding, which is consistent with a high level of transmission within a herd (Chase-Topping *et al.*, 2007).

Cross contamination of hides and hooves with STEC can occur pre-slaughter during transport to the slaughter house or in holding pens. A positive association was found between cattle carcasses testing positive for *E. coli* O157:H7 and transportation in a truckload which contained at least one high shedding (>5x10^4 CFU/g of faeces) cow (Fox *et al.*, 2008). In this study, carcasses were sampled post hide removal but before evisceration or post-harvest interventions.

2.4 Exposure Assessment

KEY FINDINGS

E. coli carrying the *stx1* and/or *stx2* genes have been identified in up to 15% of samples in surveys of retail red meats in New Zealand. However, the prevalence of STEC isolates which contain virulence genes in addition to the *stx* genes is much lower (<2%). The data from the most recent New Zealand survey indicate that STEC are present in red meats at very low concentrations, up to 3.3 MPN/g.

Since the previous Risk Profile, many studies have been undertaken to assess the carriage of STEC by red meat livestock in New Zealand. STEC have been shown to occur in cattle, dairy cows, sheep and lambs, with a higher prevalence found in very young calves. As with red meat, the prevalence of samples containing *stx* genes is much higher than the prevalence of isolates containing additional virulence genes, or serotypes associated with human cases. No studies have been undertaken to examine the prevalence of STEC in live pigs or deer in New Zealand.

Red meats are commonly consumed products with approximately 69% of respondents aged 15 years or over in the 2009 Adult Nutrition Survey reporting consumption of red meat (beef, veal, sheep meat, pork and venison) during the 24 hours prior to the survey.

2.4.1 New Zealand prevalence and frequency studies

The New Zealand Ministry for Primary Industries has been monitoring beef and veal meat for the presence of *E. coli* O157:H7 since 1998. In the second quarter of 2012, the STEC testing programme for red meat was expanded to include six additional serotypes: O26, O45, O103, O111, O121, and O145 (see section 5.1.2). Sampling is conducted at meat processing plants that export part or all of their production, and results are reported to the National Microbiological Database (NMD) administered by the Ministry. These results are not publicly available.

2.4.1.1 STEC in meat: O157:H7

A survey of retail raw meats (minced or diced samples) was undertaken between 2003 and 2005 which tested 233 beef, 183 unweaned veal, 231 lamb/mutton and 231 pork samples for *E. coli* O157:H7 (Wong *et al.*, 2006). The prevalence of STEC was determined by PCR for *stx1* and *stx2* on an enrichment broth. The *stx1* and *stx2* genes were found in 12/233 beef samples (5.2%, 95% CI 2.7-8.8%), 34/231 lamb/mutton samples (14.7%, 95% CI 10.4-20.0), 15/231 pork samples (6.5%, 95% CI 3.7-20.55) and 4/183 samples of unweaned veal (2.2%, 95% CI 0.6-5.5%). The results for all serotypes from this survey are shown in Appendix 1. Further analysis to identify positive samples containing *E. coli* O157:H7 involved immunomagnetic separation (IMS) and various confirmatory tests on isolates. *E. coli* O157:H7 (carrying *stx1* and/or *stx2*, *eae* and *hlyA* genes) was isolated from none of the beef samples

(0/233), 1 unweaned veal sample (1/183, 0.5%, 0-3.0% 95% CI), 3 lamb/mutton samples (3/231, 1.3%, 0.3-3.7% 95% CI) and 1 pork sample (1/231, 0.4%, 0-2.4% 95% CI). The concentration of the pathogen was estimated by MPN analysis and determined to be at <0.33 MPN/g for the positive pork, unweaned veal and one of the lamb/mutton samples, 1.0 and 3.3 MPN/g for the remaining two lamb/mutton samples.

A study to assess the likelihood of the introduction of novel pathogen strains into New Zealand (Wong *et al.*, 2009) tested 100 New Zealand produced (domestic) pig carcasses and 110 imported pig meat samples over an 8 month period. Swabs or excised samples were enriched and tested for *E. coli* O157:H7 using a commercial immunoassay. Positive enrichments were further analysed using IMS and confirmatory tests on presumptive isolates. One isolate of *E. coli* O157:H7 containing virulence genes was identified from domestic pig carcasses, and two from imported meat. The prevalence of *E. coli* O157:H7 on domestic pig carcasses was 1% (95% CI 0.03-5.4) while the prevalence on imported pig meat was 1.8% (95% CI 0.2-6.4). The two positive isolates from imported meat were detected in meat from Australia. The remaining imported samples, from Canada and the USA, were all negative. It should be noted that at the time of this survey imported pig meat from these two countries had to be cooked before release onto the New Zealand market. This survey did not attempt to identify non-O157 serotypes.

2.4.1.2 STEC in meat: Non-O157 STEC

The study of retail raw meat described in the previous section also examined the presence of non-O157 STEC (Wong *et al.*, 2006). Each of the sample enrichments positive for STEC yielded one *stx* containing serotype. However, in addition to the O157:H7 serotypes, only two O26:H11 isolates from two different unweaned veal samples had the full complement of virulence genes (*eae*, *hlyA*, and *stx1* or *stx2*) (2/183, 1.1%, 95% CI 0.1-3.9%). Counts of STEC were low (up to 3.3 MPN/g).

A study of beef trim imported from Australia, New Zealand and Uruguay into the US has been conducted, to provide data to compare with that from US sourced meat (Bosilevac *et al.*, 2007). Tests were conducted for indicator bacteria and several pathogens. Meat from New Zealand had the lowest prevalence (23/233 samples; 9.7%) of *stx* genes detected by PCR, compared to 30% each for Australia, and the USA, and 28% for Uruguay. Samples positive for *stx* genes were then further processed to isolate the source of the genes. Only non-O157 STEC were the subject of these analyses. STEC were isolated from four of the 23 positive New Zealand samples (1.7%, O26:H8, O26:H11, O64:H9, O163:H19), and two of these were described as HUS related types. Overall, it was concluded from all the testing, that Australian and New Zealand beef trim had lower levels of contamination than US beef trim.

2.4.1.3 STEC among livestock

Young calves are more likely to carry *E. coli* O157:H7 due to the developing nature of their gut microflora. In a study of calf processing at nine premises in New Zealand, 17/160 (11%) of faecal samples and 69/160 (43%) of young calf hides at the opening cut line were positive for *E. coli* O157:H7, suggesting that contamination by multiple faecal sources occurs on hides (Mills *et al.*, 2006). During processing 17/200 (9%) of carcasses were positive; after three different interventions to control bacterial contamination (Inspexx 200^{TM} carcass wash, acidified sodium chlorite of opening cut lines, steam vacuum of opening cut lines), the prevalence reduced to 13/200 (7%). The concentration of the organism reduced during processing: from around 0 $\log_{10}$ MPN/cm^2 (range -1 to 2) on hides, to -1 $\log_{10}$ MPN/cm^2 (range

-2.5 to 0) on carcasses pre-intervention, to -1.5 $\log_{10}$ MPN/cm^2 (range -2.5 to -1) on carcasses post-intervention.

Isolates of *E. coli* from recto-anal mucosal swabs obtained from 187 cattle and 132 sheep in the Manawatu and Rangitikei regions of New Zealand have been serotyped (Cookson *et al.*, 2006c). Of the 319 animals tested 43% harboured *E. coli* isolates possessing *stx* and 33% *eae*. Eleven different O serotypes were identified among the isolates; O5:H-, O9:H51, O26:H11, O65:H-, O75:H8, O84:H-, O84:H2, O91:H-, O128:H2, O149:H8, O150:H8 and O174:H8. Of these, 6 serotypes have been reported as isolated from human cases of gastrointestinal disease internationally and 5 with cases of HUS. No O157 serotypes were discovered in this study, but the analytical methodology employed was generic for *E. coli* and not selective for O157. This study confirms that cattle and sheep in New Zealand are major reservoirs of STEC in New Zealand.

PCR analysis of 952 isolates from the 319 animals tested in the above dataset has also been performed (Cookson *et al.*, 2006b). Ninety nine isolates (10.4%) were positive for *stx1* only, 83 (8.7%) were positive for *stx2* only, 33 (3.55) were positive for *stx1* and *stx2*, and 115 (12.1%) were positive for *eae* only. The proportions of STEC which would be classed as positive under the NMD protocol were 23 (2.4%) *stx1* and *eae*, and 1 (0.1%) *stx2* and *eae*.

The isolation of STEC O84 from beef and sheep in New Zealand has been reported (Cookson *et al.*, 2006a). Nine beef isolates and two sheep isolates from New Zealand animals were examined, along with five human isolates. The study noted some similarities between STEC of this serotype isolated from ruminants and human cases in New Zealand, although the types did not have indistinguishable PFGE profiles.

A survey of dairy cow faeces on four farms across New Zealand from 2005 to 2006 identified STEC in 2/155 (1.3%) samples (Moriarty *et al.*, 2008). One isolate was identified as *E. coli* O130:H11, possessing the *stx1*, *eae*, and *hlyA* genes. From 2004 to 2013 this serotype has been isolated from one human case in New Zealand (in 2008). The second isolate was provisionally identified as H38, as the O serogroup could not be typed. Although this result is most relevant to milk contamination, Bobby calves come from the same environment, and dairy cows are also slaughtered for human consumption (Buncic and Avery, 1997).

A study of lamb faecal samples at slaughter (December 2006-February 2007) and sheep faecal samples at pasture (April 2008-March 2009) has been performed (Moriarty *et al.*, 2011). STEC were isolated from 4/105 (3.8%) of lamb faecal samples and 2/220 (0.9%) of sheep faecal samples. The STEC-positive isolates obtained from lambs were identified as *E. coli* O176:HNM and *E. coli* O157:H7 (2 each), and those from sheep were both identified as *E. coli* O128:HNM.

The results of testing of faecal samples from very young calves (4-7 days old) and adult cattle processed at New Zealand slaughter plants from 2009 to 2011 for *E. coli* O26 have been reported (Cookson *et al.*, 2012). Each isolate was screened for genes associated with virulence, as well as typing by PFGE and Stx-encoding Bacteriophage Insertion (SBI) methods. In adult cattle the prevalence of *E. coli* O26 was 4/883 (0.5%) and in young calves 29/695 (4.2%). Only *stx1* genes were found in positive isolates. Despite the widespread occurrence of this serogroup in these animals, few cases of human infection have been identified (6 cases during 2004-2013).

Further studies describe various approaches to the determination of STEC prevalence rates in samples from very young calves. One study was focused on STEC O157 alone (Irshad *et al.*, 2012a; Irshad *et al.*, 2012b), and detected real-time PCR products consistent with the presence of STEC O157 in 55 of 309 (17.7%) recto-anal mucosal (RAM) sample enrichments. However, following immunomagnetic separation (IMS) and plating of PCR positive samples only 10 samples (3.2%) were positive; seven with the gene composition *stx2*, *eaeA* and *ehxA* and three with *stx1*, *stx2*, *eaeA* and *ehxA* (N.B. This assumes that the 10 positives were from different animals as the 10 are referred to as both "isolates" and "samples" in the paper).

Two further papers report results from a set of 299 samples collected from two North Island processing plants. The process followed in one paper (Irshad *et al.*, 2012c) was similar to that in the preceding paragraph, except that the method was focused on STEC O26, O103, O111 and O145. None of the 299 enrichments was positive for STEC O111, while 137 (45.8%), 68 (22.7%) and 47 (15.7%) were positive for STEC O26, O103 and O145 respectively. After IMS and plating of PCR positive samples, 49 (16.4%), 5 (1.7%) and 5 (1.7%) were positive for these three serotypes. Twenty five of the 49 O26 positive samples were STEC; the remaining isolates did not contain a *stx* gene. Only one of the five O103 colonies was STEC and none of the O145 isolates contained a *stx* gene.

In the most recent paper (Irshad *et al.*, 2014) the enrichment samples were plated to two different media, with up to four isolates from each sample tested (one each of β-glucuronidase positive/negative and sorbitol fermenting positive/negative). STEC was detected in only 8 of the samples (2.7%), and atypical EPEC in 37 (12.3%) of the samples. One of the STEC isolates only contained the *stx2* gene (ONT:HNM). Of the seven isolates with *stx1* or *stx2* and *eaeA* and *ehxA*, three were STEC O157, two were STEC O26, one STEC O71:HR, and one O68:H24.

2.4.2 Food consumption: Red meat

Since the previous Risk Profile, the available data indicate that red meat continues to be consumed by a high proportion (69%) of the New Zealand population on a daily basis. There are some data that indicate a modest reduction in the prevalence of beef consumption.

The primary sources of food consumption data in New Zealand are national nutrition surveys. Specifically, the 1997 National Nutrition Survey (NNS, people >15 years) (Russell *et al.*, 1999), the 2002 National Children's Nutrition Survey (CNS) (Ministry of Health, 2003), 2003) and the 2009 Adult Nutrition Survey (ANS, people aged >15 years). Individual record data from the latter have recently been analysed (Cressey, 2013). The following data are extracted from that report, and reflect respondents' consumption in the preceding 24 hour period.

Table 2: 2009 ANS data on meat consumption by adults in New Zealand from 24 hour dietary recall records by 4721 respondents

	Beef	Sheep meat	Pork	Venison
Number of servings	2752	869	2295	18
Number of consumers (percentage of total respondents)	2171 (46.0%)	769 (16.3%)	1807 (38.3%)	17 (0.4%)
Servings/consumer/day	1.3	1.1	1.3	1.1
Consumer mean (g/person/day)	106.8	79.7	71.8	146.1
Population mean (g/person/day)	49.1	13.0	27.5	0.5
Mean serving size (g)	84.3	70.5	56.6	137.9
Median serving size (g)	56.3	35.2	31.2	111.0
95th percentile serving size (g)	242	227.5	185.0	288.8

There has been a slight, statistically significant (P<0.05), decline in the prevalence of beef consumption by New Zealand adults since the 1997 NNS survey, although this is counteracted to some extent by larger serving sizes and higher daily intakes. While there has not been any change in the prevalence of consumption of sheep meat, mean serving sizes do appear to have increased, but the difference is not statistically significant (P=0.18). While the prevalence of pork consumption has remained unchanged from 1997 to 2009, average serving sizes have increased significantly (P<0.05). Given the diversity of meat products containing pork, this could reflect either a general increase in serving sizes or a move from pork products with typically smaller sizes to pork products with typically larger sizes.

Analysis of data from the 2002 National Children's Nutrition Survey (Ministry of Health, 2003) indicated 51%, 17% and 37% of children consumed beef, sheep meat and pork respectively during any 24 hour period. Mean serving sizes for beef, sheep meat and pork for New Zealand children are 64 g, 60 g and 39 g, respectively (Cressey *et al.*, 2006).

2.4.2.1 Consumption of red meat – longitudinal data

Systematic longitudinal information on meat consumption by New Zealand is available from Statistics New Zealand summaries occasionally published by Meat and Wool New Zealand's Economic Service and from Food Balance Sheets (FBS) consolidated by the Food and Agriculture Organization of the United Nations.[10] Table 3 gives data from these two sources showing trends in meat consumption in New Zealand since 1985 (Food Balance Sheet data are provided up to 2009, the most recent available year).

Table 3: Meat consumption in New Zealand (kg/person/year), 1985-2009

Meat type	Year

[10] Food Balance Sheet data from http://faostat3.fao.org/ accessed 28 June 2013

	1985	1995	1999	2001	2003	2006	2007	2009
Statistics New Zealand data								
Beef	36.5	34.6	31.2	27.1		34.3		
Mutton	20.9	12.7	6.6	6.9		3.1		
Lamb	6.4	10.5	7.7	9.7		9.9		
Pork	14.2	15.7	17.1	16.5		19.6		
Poultry	15.0	26.2	26.8	31.0		36.5		
Food Balance Sheet data								
Bovine meat	49.3	45.5	33.8	25.9	26.5	25.8	32.1	27.6
Sheep meat	27.3	34.7	28.6	24.7	24.8	23.2	23.3	23.2
Pork	8.6	15.8	18.8	17.7	20.7	21.8	22.9	21.8
Poultry	14.3	23.9	25.4	30.6	35.2	35.2	34.7	30.4

While the two data sets show somewhat different trends, there are indications of a decrease in consumption of beef and an increase in consumption of pork and poultry since the earliest data from 1985. The most recent data, since the previous Risk Profile in 2002, indicate that consumption of red meat has been relatively stable, which is in agreement with the nutrition surveys.

2.5 Overseas Context

2.5.1 Prevalence and frequency studies in other countries

2.5.1.1 E. coli O157:H7

Numerous investigations have determined the prevalence of STEC in beef cattle, both adults and calves, but it is difficult to compare results due to the variation in sampling and analytical methods. Reported rates in the literature vary considerably from 0% to up to 70% (Gyles, 2007). Reviews of published reports of the prevalence of STEC in beef cattle have found that in general the prevalence of *E. coli* O157:H7 ranged from 0.3 to 19.7% in feedlot cattle (Argentina, Czech Republic, USA), from 0.7 to 27.3% in cattle on irrigated pasture (Brazil, Canada, Japan, Norway, USA) and from 0.9 to 6.9% in cattle grazing on rangeland forages (USA) (Hussein and Bollinger, 2005; Hussein, 2007). The same reviews have reported the prevalence of non-O157 STEC to range from 4.6 to 55.9% in feedlot cattle, and from 4.7% to 44.8% in grazing cattle.

Surveys of the prevalence of *E. coli* O157:H7 in meat products (mainly minced beef) have been undertaken in Argentina, Ireland, France, Italy, Morocco, Palestine and the USA. The prevalence of *E. coli* O157:H7 in meat is essentially similar to New Zealand with a prevalence of up to 5% for *E. coli* O157:H7 (Appendix 1, Table 9). The prevalence of all STEC is markedly higher but the pathogenicity of these isolates is uncertain.

Some quantitative data are available for products involved with outbreaks or cases caused by *E. coli* O157:H7 and for general testing of food samples. This information is presented in Table 10, Appendix 4. The counts obtained were generally low (<5 MPN/g) but occasionally high counts were detected (>100 CFU/g).

2.5.1.2 Non-O157 STEC

Worldwide the prevalence of non-O157 STEC in beef cattle has been reported as from 2.1-70%. Approximately 193 serotypes have been reported from dairy and 261 serotypes from

beef cattle and 12-17% of these serotypes have been implicated in human illness. Many of the apparently non-pathogenic strains appear to be lacking one or more virulence factors associated with disease (Hussein and Bollinger, 2005).

An Irish study tested cattle faecal samples (n=1200) and farm soil samples (n=600) from 20 farms over 12 month period for non-O157 STEC (Monaghan *et al.*, 2011). Of the faecal and soil samples, 40% and 27% were *stx* positive respectively with an increase in prevalence observed in late summer-early autumn. The 107 STEC isolates recovered represented 17 different serotypes. O26:H11 and O145:H28 were most clinically relevant isolates found, while O113:H4 was the serotype most frequently isolated. However, serotypes O2:H27, O13/O15:H2 and ONT:H27 were also identified amongst the isolates, and these carried *stx*1, and/or *stx*2 and *eae*A genes. Consequently they were classed as possibly emerging pathogens.

A survey of non-O157 STEC in commercial ground beef samples (n = 4,133) obtained from numerous manufacturers across the US over a period of 24 months (July 2005 to June 2007) has been reported (Bosilevac and Koohmaraie, 2011). All samples were screened for the presence of Shiga toxin genes, which were present in 1,006 (24.3%) of the samples. Of the 1,006 positive ground beef samples screened for *stx*, 300 (7.3% of the total of 4,133) were confirmed to have at least one strain of STEC present by culture isolation. In total, 338 unique STEC isolates were recovered from the 300 samples that yielded an STEC isolate. All unique STEC isolates were serotyped and were characterized for the presence of a range of genetic virulence factors. Results of this characterization identified 10 STEC isolates (0.24% of the 4,133 total) that were considered a significant food safety threat, defined by the presence of genes associated with virulence factors (*stx1*and/or *stx2*, *eae*, and a gene for a subtilase cytotoxin *subA*).

2.5.2 Red meat consumption in other countries

Table 8 in Appendix 1 shows comparisons of red meat consumption rates for the year 2003 for a range of countries with high meat consumption, taken from Food Balance Sheets.

According to these data, New Zealanders are high, but not the highest, consumers of red meat worldwide.

3 EVALUATION OF ADVERSE HEALTH EFFECTS

3.1 Disease Characteristics

KEY FINDINGS

Infection by STEC in humans usually results in bloody diarrhoea (haemorrhagic colitis), and a small proportion of patients (usually less than 5%) will go on to suffer more serious outcomes including haemolytic uraemic syndrome (HUS) which results in acute kidney damage, and in 1-4% of HUS cases death (Spinale *et al.*, 2013). While 70% of patients with HUS recover completely, the remainder suffer a range of long term sequelae primarily related to chronic renal disease (proteinuria (15-30% of cases); hypertension (5-15%); chronic kidney disease (CKD; 9-18%); and end-stage kidney disease (ESKD; 3%)), and less frequently sequelae such as gastrointestinal complications, neurological disorders and diabetes mellitus (Croxen *et al.*, 2013; Spinale *et al.*, 2013).

New information from overseas shows that the proportion of cases of STEC infection that develop HUS (up to 10%) is higher than previously estimated. Infection with O157 STEC is more likely to result in the development of severe illness compared with infection by non-O157 STEC.

New information has been published on haemolytic-uraemic syndrome (HUS), which is a serious sequelae of STEC infection (HUS can also be caused by infection by *Shigella dysenteriae* type 1). A recently-published systematic review of published case-control studies on HUS found that 61% of HUS cases may be attributable to a previous infection with STEC (Walker *et al.*, 2012). In a recent analysis of reported STEC cases in the EU, 10% developed HUS (EFSA, 2013), while an analysis of reported *E. coli* O157:H7 infection in US FoodNet sites found HUS resulting from 6.3% of reported cases (Gould *et al.*, 2009) (note that these percentages would be lower if unreported cases of STEC infection were included in the denominator). These proportions are higher than the 4% previously estimated (Mead *et al.*, 1999). An analysis of ten years' of epidemiological data in the USA found that bloody diarrhoea, hospitalisation, and HUS were more common in patients infected by O157 STEC than in patients infected by non-O157 STEC (Hadler *et al.*, 2011).

3.2 Dose Response

KEY FINDINGS

There is no known safe level of exposure for ingestion of *E. coli* O157:H7. Based on a model derived from outbreak data, a dose of 100 cells provides a median 50% probability of infection, while 10 cells provides a median 20% probability of infection (Strachan *et al.*, 2005). Dose-response data are for *E. coli* O157:H7 specifically; dose response models for non-O157 STEC have not yet been established.

Dose response information can be presented as a definitive number of cells that cause infection (infectious dose) or the probability of infection by exposure to differing numbers of cells. There is a trend towards the latter approach.

3.2.1 Infectious dose

No new data on infectious dose were located. An investigation of a 2005 outbreak of *E. coli* O157:H7 infection in the USA caused by contaminated raw milk found that the risk of illness increased with an increasing number of cups of milk consumed daily, but the concentration of *E. coli* O157:H7 in the milk consumed in this outbreak was not reported (Bhat *et al.*, 2007; Denny *et al.*, 2008).

There are few data on which to base a dose-response relationship for non-O157 STEC. A summary of available information was prepared for a USDA Risk Profile on non-O157 STEC (FSIS, 2012). This report commented that the minimum dose estimates for STEC serogroups O111 and O145 appeared to be comparable to minimum dose estimates for *E. coli* O157:H7.

3.2.2 Probability of infection

A paper examining Beta-Poisson dose response models for *E. coli* O157:H7 was published in 2005 (Strachan *et al.*, 2005). The data were derived from eight outbreaks of *E. coli* O157 infection in the UK, USA, and Japan. The best fit was found for the exact Beta-Poisson with beta-binomial likelihood model, which provided a curve which estimated that a dose of 100 cells provides a median 50% probability of infection, while 10 cells provides a median 20% probability of infection.

A dose-response model which estimates the probability of the sequela HUS from exposure to *E. coli* O157 has been published (Delignette-Muller *et al.*, 2008; Giacometti *et al.*, 2012). This model predicts the probability of HUS directly from the ingested dose, without considering the intervening infection step.

3.3 New Zealand Human Health Surveillance

KEY FINDINGS

Food associated risk factors, including eating meat, were not identified as risk factors for STEC infection in a recent case control study. Red meat has not been confirmed as the source of any outbreaks of STEC infection in New Zealand.

The annual rate of reported STEC infection rose from 2006 to 2009, was relatively stable from 2009 to 2012, and rose again in 2013. The highest age-specific rates of infection continue to be observed in young children. Up to 43% of cases of STEC infection were hospitalised in the years 2006 to 2013, and one death was reported in 2009. *E. coli* O157:H7 continues to be the predominant serotype isolated from New Zealand cases of STEC infection. However, New Zealand clinical laboratories do not have routine protocols and procedures in place for detecting non-O157 STEC and there are no national testing protocols for the isolation of non-O157 STEC.

3.3.1 STEC infection in New Zealand

The rate (per 100,000 population) of STEC infection increased from 1.3 in 1998 to 2.5 in 2005, and between 2009 and 2012 was between 3.0 and 3.5 (Table 4). The 2013 rate rose to 4.6 per 100,000, with the increase due to a larger number of *E. coli* O157:H7 cases, mainly during the autumn period (Horn *et al.*, 2014). Changing laboratory protocols will only account for a small part of this rate increase, since a survey of New Zealand clinical laboratories in 2010 found

that most had not changed their isolation method since 2006 (Nicol *et al.*, 2010). Importantly, laboratory methods are biased toward detecting *E. coli* O157, so cases of non-O157 STEC infection are likely to be underreported in public health surveillance data.

STEC infection can affect any age group but most reported disease is in children aged four years or less. The reported rate per 100,000 was elevated each year in very young children (Table 4); with the lowest notification rates in the 30-60 year age group. Notification rates for very young children are affected by the fact that parents are more likely to seek medical advice for infants than older children (Skirrow, 1987).

There were regional differences in annual rates. Notably, the Waikato District Health Board region was among those with the highest rates every year. The high rates in the Waikato region may be associated with the high number of cattle in the district.[11] Notification rates tend to follow a seasonal pattern with peaks in late summer/autumn and spring. The annual rates of STEC infection between 2006 and 2012 were generally similar for males and females, while in 2013 females had a higher rate of notification (rate 5.3 vs 3.9 for males).

A large number of STEC cases go unreported each year. An estimate of the total number of reported and unreported cases of gastroenteritis caused by STEC infection for 2005 was 340 (95% CI 180-620) cases per year (Cressey and Lake, 2007). A subsequent estimate of 2,830 cases (95% CI 120-10,500) was calculated using alternative multipliers from overseas studies (to adjust reported cases to total cases) and notification data from 2011 (Cressey, 2012). Roughly, this means that for every case that is reported, 17 are not. This is equivalent to a rate of 70.8 per 100,000, which is higher than a recent Australian estimate, but similar to recent estimates for the USA and Canada (see Appendix 2).[12] The author of the 2012 document stressed that there was a high level of uncertainty in the multipliers (as reflected in the confidence intervals for the estimated number of cases).

While the number of reported STEC cases is small compared to other notifiable diseases, the clinical outcomes are often severe. For the period 2006-2013, the hospitalisation status was known for over 80% of STEC cases each year, and the proportion of these cases hospitalised per year was in the range 28-43%. One death was reported in 2009, which is the first death reported since 1998.

Between 2003 and 2012, the number of hospitalised cases of HUS ranged from 20 to 39 per year, with 50 occurred in 2013 (Horn *et al.*, 2014; Lopez *et al.*, 2013). These data do not specify whether STEC was the primary cause of HUS, but Table 4 also includes data from the New Zealand Paediatric Surveillance Unit (NZPSU) of the number of reported HUS cases from which STEC were isolated. The number of such cases also increased markedly in 2013. Also worth noting is that all cases of paediatric HUS in Table 4 from which STEC were isolated were found to be *E. coli* O157:H7, except for one in 2013 which was *E. coli* O179:H8.

Table 4: Notified cases of STEC infection in New Zealand, 2006-2013

[11] Statistics for the 2011/12 year showed that the Waikato region contained almost a quarter (24.6%) of all New Zealand's dairy cows (LIC, 2012).. The next highest was the Taranaki region (10%).
[12] Rate per 100,000 calculated using the estimated New Zealand resident population mean for the year ending 2011 of 4,407,400 (http://www.stats.govt.nz/infoshare accessed 7 August 2013).

Year	Total number of cases	Rates of STEC infection per 100,000				Reference
		All cases	Cases aged <1 year	Cases aged 1-4 years	No. reported paediatric cases of HUS*	
2006	87	2.1	10.2	16.7	12	(Pirie *et al.*, 2008)
2007	100	2.4	19.4	18.2	4	(Williman *et al.*, 2008)
2008	128	3.0	7.8	16.5	3	(Williman *et al.*, 2009)
2009	143	3.3	14.3	21.9	4	(Lim *et al.*, 2010)
2010	138	3.2	14.1	25.8	4	(Lim *et al.*, 2011)
2011	154	3.5	14.4	23.8	6	(Lim *et al.*, 2012)
2012	147	3.3	14.9	22.7	3	(Lopez *et al.*, 2013)
2013	207	4.6	20.0	26.6	11	(Horn *et al.*, 2014)

*These data are reported annually by the New Zealand Paediatric Surveillance Unit, and are the number of reported cases of paediatric HUS with diarrhoea from which STEC were isolated. Child normally implies an upper age limit of 15 years.

3.3.2 Case control studies and risk factors

Risk factor information from sporadic cases of STEC infection are summarised in the annual reports on foodborne disease in New Zealand published by MPI.[13] In the report for 2013, consumption of red meat was reported by a high proportion of cases (beef products 77.4%, lamb products 38.1%, pink or undercooked meats 8.7%). These proportions were similar to previous years. In both 2012 and 2013 risk factors reported by a higher proportion of cases were consumption of raw fruit/vegetables (93.9%), contact with household pets (87.1%), and consumption of dairy products (87.7%). Consumption of home killed meat was reported as a risk factor in 32.5% of cases. The report also notes that the reporting of exposure to a risk factor does not imply that this was the source of the infection, and these risk factors are common behaviours in New Zealanders' lives with a median of 4 (range 2-7) risk factors reported per notification. Risk factor information was only obtained for 45-55% of STEC notifications in 2013.

Analysis of the 2012 data, showed that 97/147 (66%) notifications had a residential address located in an urban area[14] compared to 50/147 (34%) located in a rural area. This equates to a rate of 2.5 notifications per 100,000 for the urban population, compared to 8.1 notifications per 100,000 for the rural population[15]. Of the 97 urban notifications, 20 listed as one of their potential risk factors farm visits or contact with farm animals or their faeces. Other factors included in the notification records included drinking water supplies which may not be

[13] http://www.foodsafety.govt.nz/science-risk/human-health-surveillance/foodborne-disease-annual-reports.htm accessed 9 July 2013

[14] Urban area defined by Main Urban, Independent Urban or Satellite Urban Area as defined by Statistics NZ. Rural areas are the remainder of those areas defined in the urban rural classification. http://www.stats.govt.nz/browse_for_stats/people_and_communities/Geographic-areas/urban-rural-profile.aspx accessed 27 February 2014

[15] Urban-Rural population estimates for 2012 were sourced from http://www.stats.govt.nz/~/media/Statistics/browse-categories/people-and-communities/geographic-areas/urban-rural-profile-update/population-estimates.xls, accessed 27 February 2014

monitored or treated, recreational water activities in rivers, lakes or the sea, drinking of unpasteurised milk or person to person transmission.

An examination of historical human isolates from the Enteric Reference Laboratory (2000-2010) and isolates collected from very young calves from four processing plants (2009-2011) has found significant differences in the geographical distribution of both human and bovine *E. coli* O157 genotypes (Jaros *et al.*, 2012). A total of 207 human and 28 bovine isolates from the North and South Islands were analysed. All isolates were *hlyA*, *eae* and *stx2* positive, and 14% were also positive for *stx1*. Two human isolates were *stx2* negative but *stx1* positive. There was evidence of a different pattern of genotypes between the North and South Islands. Stx encoding bacteriophage insertion (SBI) typing showed that amongst human isolates SBI type 5 was most common in the South Island while SBI type 1 was most common on the North Island. Amongst the bovine isolates, all North Island isolates were SBI type 1 or 3, while all South Island isolates were SBI type 5.

A prospective case-control and molecular epidemiological study of human cases of STEC infection in New Zealand was conducted from July 2011 to July 2012 (Jaros *et al.*, 2013). Questionnaire data from 113 eligible cases and 506 controls were analysed using multivariate logistic regression. Statistically significant animal and environmental risk factors were identified. In particular, "cattle present in meshblock", "contact with animal manure", and "contact with recreational waters" were all significant risk factors. Food associated risk factors, including eating meat, were not identified as risk factors for increased risk of STEC infection in univariate or bivariate regression analysis. However, the modest number of cases in this study may have made it difficult to identify risk factors with lower elevated risk.

3.3.3 Reported outbreaks

Outbreaks of STEC infection continue to make up only a small proportion of the total reported outbreaks and outbreak-associated cases each year in New Zealand (Table 5); note that percentages in this table are reported for all enteric outbreaks, not all outbreaks as was reported in the previous Risk Profile, although the difference is minimal). From 2006 to 2013, 6/43 of the outbreaks of STEC infection were reported as foodborne, but red meat was not implicated as the vehicle of infection for any of these outbreaks.

Table 5: Reported outbreaks of STEC infection in New Zealand and information on those reported as foodborne (2006-2013)

Year	Number of STEC outbreaks (% of all reported enteric outbreaks)*	Number of. cases associated with STEC outbreaks (% all cases associated with enteric outbreaks)	No. foodborne STEC outbreaks	Food(s) implicated (level of evidence)	References
2006	5 (1.0%)	16 (0.3%)	0	N/A	(Pirie *et al.*, 2008)
2007	6 (1.3%)	13 (0.2%)	2	4 confirmed cases. No food vehicle implicated.	(Williman *et al.*, 2008)
2008	4 (0.9%)	25 (0.4%)	1	14 cases. Vehicle not confirmed.	(Williman *et al.*, 2009)
2009	4 (0.7%)	15 (0.1%)	0	N/A	(Lim *et al.*, 2010)
2010	5 (0.9%)	12 (0.2%)	1	3 cases. Suspected vehicle was undercooked chicken.	(Lim *et al.*, 2011)
2011	2 (0.4%)	7 (0.1%)	0	N/A	(Lim *et al.*, 2012)
2012	1 (0.2%)	3 (<0.1%)	0	N/A	(Lopez *et al.*, 2013)
2013	16 (2.5%)	58 (0.8%)	2	Raw milk for one outbreak, and no food vehicle identified for the other.	(Horn *et al*., 2014)

* From 2006 to 2008 outbreaks were reported as VTEC/STEC. In 2009 outbreaks were reported as *E. coli* O157, in 2010 and 2011 outbreaks were reported as *E. coli* O157:H7, and in 2012 outbreaks were reported as VTEC/STEC.

3.3.4 Serotypes

The 2002 Risk Profile reported that *E. coli* O157:H7 was the predominant serotype isolated from STEC cases in New Zealand. This pattern has continued, although the proportion of non-O157 isolates associated with human disease is possibly increasing (Table 6). Changing laboratory procedures might account for this increase. A 2010 report found that New Zealand laboratories did not have consistent protocols and procedures in place for detecting non-O157 STEC and that there were no national testing protocols for the isolation of non-O157 STEC in New Zealand (Nicol *et al.*, 2010).

Table 6: STEC serotypes identified by ESR's Enteric Reference Laboratory, 2009-201[1]

Serotype	2009	2010	2011	2012	2013
O157	**137**	**115**	**139**	**119**	**192**
O157:H7	137	115	139	119	192
Non-O157	**8**	**13**	**14**	**23**	**22**
O121:H19					2
O128:H2		1	2		1
O84:H2		1	2		
O176:HNM		2	1	1	
ONT:HNM	3			9	1
ONT:H11				2	1
Other types[a]	5	9	9	11	17
Unable to be typed					
Total	**145**	**128**	**153**	**142**	**215**

[a] Single cases following types were identified

2009: O22:H16, O103:H25, O174:H21, O26:H11, O103:H2

2010: ONT:H21, ONT:H23, ORough:HNT, ORough:H7, O77:HNM, O123:H8, ONT:HRough, O68:HNM, ONT:H2

2011: O103:H2, O123:HNM, O131:HRough, O146:H21, O178:H23, O26:H11, O84:HNM, ONT:H2, ORough:H2

2012: O26:H7, O26:H11, O38:H26, O68:HNM, O84:HNM, O128:HNM, O146:H21, O146:HRough, O176:HRough, O180:HNM, ONT:H7

2013: O26:M11, O38:H26, O84:HNM, O84:HNT, O103:H25, O116:H11, O121:HNT, O123:HMN, O145:H34, O156:H25, O163:H19, O177:HNM, O179:H8, O182:HNM, ONT:H2, ORough:H2, ORough:HNM.

3.4 STEC Infection Overseas

KEY FINDINGS

Reported rates of STEC infection per 100,000 for 2011-2012 in developed countries ranged between 0.1 and 9.0 per 100,000 population. Most countries, including Australia, had reported rates lower than New Zealand. The proportion of isolates serotyped as being non-O157 has increased for most countries, which is similar to the situation in New Zealand. Of note is that only 59% of STEC isolates serotyped in Australia in 2010 were O157.

Appendix 2 contains detailed data summarised in this section.

Red meat has been confirmed as a vehicle in outbreaks of both *E. coli* O157:H7 and non-O157 STEC infections overseas (Appendix 2 Table 12 and Table 13). Case-control studies have also linked red meat consumption and STEC infection in a number of countries (Appendix 2 Table 14) but it is notable that most involve hamburgers and sausages, where mincing and mixing will distribute any contamination.

4 EVALUATION OF RISK

4.1 Existing Risk Assessments

There has been no quantitative risk assessment for STEC in red meat published for New Zealand. A number of overseas risk assessments have been published for *E. coli* O157:H7 and other serotypes in meat, particularly for hamburger consumption. These have been summarised in Appendix 2. In most of these studies, the most important factor affecting risk of infection is the initial concentration of STEC in animal faeces or on meat. However, factors in the home, in particular cooking methods and the form in which meat is processed and consumed (e.g. hamburgers) are also important.

The previous Risk Profile identified the main barrier to a comprehensive risk assessment as the limited data on the prevalence and concentration of contamination by STEC of New Zealand meats at the retail level. Since then surveys have produced useful data (Wong *et al.*, 2006; Wong *et al.*, 2009). A quantitative risk model could be constructed given the available information, at least for the retail to consumption part of the food chain.

4.2 Evaluation of Risk for New Zealand

KEY FINDINGS

Since the previous Risk Profile additional studies have reported that STEC are widespread in both retail meat and livestock in New Zealand, although strains exhibiting virulence markers associated with human disease are at a lower prevalence (less than 2%). The risk of infection from red meat would be high should exposure occur, as very low numbers of cells provide a high risk of infection.

Nevertheless, information from outbreaks since 2002 and a case-control study have not provided evidence for transmission of STEC in red meat in New Zealand. Therefore the conclusion of the previous Risk Profile is unchanged, namely that "there is currently little information to suggest that transmission of STEC via red meat is occurring in New Zealand".

Cooking of red meat before consumption and hygienic food preparation are important barriers to exposure, and will mitigate the risk. Overseas outbreaks of STEC infection most often are linked to meat products where STEC are present internally, such as sausages and hamburgers, as these foods may be undercooked prior to consumption. A survey of New Zealand consumers (Gilbert *et al.*, 2007) found that no consumer preferred their sausages or minced beef/hamburgers cooked rare.

4.2.1 Risk associated with red meat and red meat products

The 2013 rate of reported STEC infection in New Zealand is higher than many comparable overseas countries at approximately 4.6 notified cases per 100,000 population. All cases have been sporadic or reported as very small outbreaks; no large outbreaks have been detected. Information on transmission routes is limited, but a recent case-control study did not find that meat consumption was a significant risk factor, and reported outbreaks in New Zealand have not been linked to red meat.

STEC serotypes associated with human disease have been isolated from the faeces of beef cattle, dairy cattle and sheep in New Zealand, signalling that contamination of meat from these species is possible. Higher prevalence has been reported for young calves.

The prevalence of the dominant disease causing serotype, O157:H7 found in surveys of retail raw meat in New Zealand is 0 - 1.3%. Data on the prevalence of non-O157 STEC in meat in New Zealand are limited, but serotypes that have been associated with serious disease (e.g. O113:H21) have been isolated from retail raw meat samples.

Very small amounts of sheep and beef meat are imported, but approximately 30% of the pig meat supply in New Zealand is imported. Therefore the risk of STEC contamination of imported pork needs to be considered. A survey has identified *E. coli* O157:H7 in both local and imported pork in New Zealand. However, pork has not been identified as a vehicle in outbreaks overseas (see Appendix 2).

No data on STEC in New Zealand venison or deer are available. An outbreak of STEC infection linked to handling and consumption of wild deer in the USA has been reported, but links between STEC infection and venison are rare (Rounds *et al.*, 2012).

The evaluation of risk from non-O157 STEC is complicated by the large number of serotypes, and the variability in the presence of genetic elements which are associated with pathogenicity (and these genetic elements are not yet fully elucidated). Given the apparent plasticity of the STEC genome, and the emergence of new pathogenic variants (such as *E. coli* O104) any classification scheme must be regarded as provisional. The identification of the "super 7" set of serotypes as adulterants by the United States Department of Agriculture is one approach. EFSA have made recommendations regarding assessment of pathogenic potential on the basis of serotypes isolated from cases of HUS. However, there appears to be a move away from classification on the basis of serotype; instead EFSA recommends determining pathogenicity not only the presence of *stx* genes, but also other genes associated with virulence (see Appendix 2 Section 8.1.2).

Risk assessment also needs to consider food related factors. STEC are normally present only on the surface of intact muscle meat, but may become internalised when meat is minced or injected. Most incidents of human infection overseas occur when STEC present in minced meat products such as sausages and hamburgers are undercooked prior to consumption. A survey of New Zealand consumers found that no consumer preferred their sausages or minced beef/hamburgers cooked rare (Gilbert *et al.*, 2007).

Information therefore now exists which shows pathogenic STEC serotypes are present in New Zealand red meat at retail, but the link between contaminated red meat and disease still has not yet been made in this country. It is likely that the stated preference of New Zealand consumers to sear whole cuts of meat and thoroughly cook ground meat products means that any STEC present on or in these products are inactivated.

4.2.2 Risks associated with other foods

An analysis of 90 *E. coli* O157 outbreaks from six developed countries found that food was the most frequently found mode of transmission (42%) and food associated outbreaks often had higher numbers of cases than those associated with other transmission vehicles (Snedeker *et al.*, 2009).

In the US, ground beef/hamburger is the food vehicle most likely to be implicated in outbreaks of *E. coli* O157:H7 (Painter *et al.*, 2013). Contaminated foods that are not cooked prior to consumption (lettuce, salads, coleslaw, spinach, spring onions) or the consumption of unpasteurised foods (milk, apple juice) have also been implicated routes of infection for outbreaks. Person to person, contact with animals and consumption of contaminated drinking water or other water sources have also been identified as transmission pathways.

4.3 The Burden of STEC Infection in New Zealand

KEY FINDINGS

On a national scale (and on the basis of existing information), the burden of disease from red meat contaminated with STEC is considered to be low due to the absence of evidence for red meat as a transmission vehicle from surveillance data and a case-control study. The burden of disease from foodborne STEC infection in New Zealand is third on a ranked list of six enteric foodborne diseases, based on an estimate from 2011.

4.3.1 Burden of disease from red meat contaminated with STEC

On a national scale (and on the basis of existing information), the burden of disease from raw meat contaminated with STEC is considered to be low due to the absence of evidence for red meat as a transmission vehicle from surveillance data and a case-control study.

4.3.2 Burden of disease from all STEC infection

An estimate of the burden of foodborne disease in disability adjusted life years (DALYs) was initially published using data principally from 2005 (Lake *et al.*, 2010), then revised using data from 2011 and multipliers from more recent overseas studies (to estimate cases not reported to the health system) (Cressey, 2012). The most recent study calculated the total burden of disease from STEC infection and sequelae as 505 DALYs, with 200 DALYs (5^{th}-95^{th} percentile 1.5-783) being foodborne. For comparison, higher DALY burden of foodborne disease estimates were for norovirus infection (873, 5^{th}-95^{th} percentile 675-1083) and campylobacteriosis (587, 5^{th}-95^{th} percentile 425-781)). The DALY estimate for foodborne STEC infection was higher than that of foodborne listeriosis, salmonellosis and yersiniosis. However, the author stressed the high level of uncertainty associated with the DALY estimates for STEC infections.

An estimate of the total economic cost to New Zealand of six foodborne diseases has been published. This estimate converted the individual burden in DALYs to an economic value and was based on data from 2009. Of the estimated total cost ($161.9m), STEC infection accounted for $14.6 million (11%), reflecting the associated risk of its rare but severe complications and premature death. This estimate was similar to those for salmonellosis ($15.4m) and listeriosis ($15.2m), but all three were below estimates for norovirus infection ($50.1m) and campylobacteriosis ($36.0m), and well above the estimate for yersiniosis ($1.9m).

4.4 Data Gaps

Although the 2002 Risk Profile did not list specific data gaps, since then a number of important pieces of information crucial to understanding the risk from STEC in red meat in New Zealand have appeared:

- Improved data not only for *E. coli* O157:H7 but also other serotypes of importance for human infections;
- Data on the prevalence of STEC in livestock;
- Data on the prevalence of STEC on red meat at retail;
- Results from a case-control study.

To supplement these data it would be useful to have information on:

- Prevalence/concentration data for STEC on intact meat (e.g. steaks, roasts) as opposed to ground/diced meat;
- The incidence of infection by non-O157 STEC, which would require routine testing by all diagnostic laboratories.

5 AVAILABILITY OF CONTROL MEASURES

KEY FINDINGS

The regulatory environment affecting the New Zealand meat industry has changed since the 2002 Risk Profile.

The majority of red meat production in New Zealand is now managed under the Animal Products Act (APA) 1999, with animal product primary processing businesses being required to have a Risk Management Programme in place by November 2002.

Overseas market access requirements have also changed, in particular for the United States. In 2008, USDA-FSIS officials announced the intention to begin testing foods for the presence of six VTEC serogroups (O26, O111, O145, O103, O45 and O121), in addition to testing for *E. coli* O157:H7. Regulatory sampling for the six non-O157 serotypes was introduced on 4 June 2012.

5.1 Current Control Measures

5.1.1 Risk Management Strategy

Although a Risk Management Strategy specifically for STEC has not been published, the Ministry for Primary Industries has an STEC science programme that is intended to identify reservoirs and sources of STECs, quantify any human health risk, and identify, implement and evaluate effective control measures. A number of research projects are reported on a dedicated website, including PFGE results and Risk Profiles.[16]

5.1.2 Relevant Food Controls

The regulatory environment affecting the meat industry has changed since the 2002 Risk Profile.

The majority of red meat production in New Zealand is now managed under the Animal Products Act (APA) 1999.[17] Requirements under this act apply to:

- primary processors (who operate under a Risk Management Programme (RMP));
- dual operator butchers (defined in section 4 of the Animal Products Act as retail butchers that also provide a homekill and recreational catch service at the same premises or place)[18]. Such businesses operate under a Risk Management Programme (RMP) guided by a dedicated Code Practice (COP);
- suppliers of home-kill, wild or game estate animals to primary processors – who must have certification; and

[16] http://www.foodsafety.govt.nz/science-risk/programmes/hazard-reduction/stec.htm accessed 17 May 2012
[17] http://www.foodsafety.govt.nz/industry/sectors/meat-ostrich-emu-game/overview.htm contains links to further detail. Accessed 1 June 2012.
[18] http://www.foodsafety.govt.nz/elibrary/industry/Dual_Operator-Requirements_Retail.htm accessed 18 June 2012

- exporters – who export meat products to countries that require official assurances (export certificates).

By 1 November 2002, all animal product primary processing businesses, except those exempt under the Act or under the Animal Products (Exemptions and Inclusions) Order 2000, were required to have a risk management programme.

Some operators may meet food safety requirements under the Food Act 1981. These include:

- secondary processors;
- people who sell meat at stalls; and
- exporters, who export beef to Australia or meat products to countries that do not require official assurances.

Operators under the Food Act need to develop a Food Safety Programme (FSP) or comply with the Food Hygiene Regulations 1974. They also need to meet the requirements of the Australia New Zealand Food Standards Code.

An RMP is a documented programme to identify and manage biological, chemical and physical hazards. The programme is based on the principles of Hazard Analysis and Critical Control Point (HACCP): identifying the hazards, the systems of control, and demonstrating that the controls are effective. RMPs are to be designed by individual businesses for the animal materials used, the processes performed and the product range produced.

A number of supporting documents for red meat processors are available at an MPI website, including:[19]

- Codex Code of Hygienic practice for meat;
- Code of Practice for Further Processing;
- RMP Models for industry sectors, including:
    - Slaughter and processing of farmed mammals
    - Slaughter and dressing of pigs
    - Slaughter, dressing, cooling and boning of sheep
    - Dual operator butchers
- Code of Practice for Processed Meats; and
- Meat at stalls Food Safety Programme.

5.1.2.1 Exporters

Red meat exporters need to comply with overseas market access requirements.[20]

As described in the previous Risk Profile, all US listed beef and sheep slaughter premises and packing houses in New Zealand participate in a mandatory microbiological monitoring programme. The results are collated by the National Microbiological Database (NMD) which is operated by the MPI on behalf of industry.

[19] http://www.foodsafety.govt.nz/industry/sectors/meat-ostrich-emu-game/documents/cops-templates.htm accessed 18 June 2012

[20] http://www.foodsafety.govt.nz/industry/exporting/ accessed 1 June 2012.

With the introduction of the requirement by the US for testing for six additional serogroups (see below) the NMD *E. coli* testing regime has been amended. As of June 2012, the Assurance GDS™ [21] system is being used to analyse enrichments from samples of beef trim (in August 2014 the separate enrichments for *E. coli* O157 and the other six serotypes were replaced by a single enrichment from which all seven serotypes can be detected). If a positive result is returned, sub-samples from the positive broth are sent to the Enteric Reference Laboratory for culture and confirmation.

5.1.2.2 Consumers

Consumer advice on STEC infection focuses primarily on *E. coli* O157:H7 but these recommendations are also valid for non-O157 strains. The current advice for the prevention of *E. coli* O157:H7 infection is outlined on the FoodSmart website through MPI.[22] Specific advice for meat is provided about cooking temperatures and prevention of cross contamination. Similar advice is also offered by the Centre of Disease Control (CDC) in the US.[23]

A study in the US observed 199 volunteers preparing hamburgers and salad and found that 22% cooked the meat below the recommended Department of Agriculture consumer end-point of 68ºC (155ºF) (Phang and Bruhn, 2011). Potential cross contamination behaviours were common, particularly through hands.

5.1.2.3 Industry controls overseas

The Food Standards Agency (FSA) in the UK undertook a consultation following a Public Inquiry report of the 2005 *E. coli* O157 outbreak in Wales[24]. This report outlined a number of recommendations, including:

- Every consumer needs to be protected from the risk of an isolated instance of low-level contamination of food by *E. coli* O157; and
- The risk to consumers from premises handling raw and Ready-To-Eat (RTE) food needs to be reduced by (1) physical separation, (2) cleaning and disinfection, and (3) hand washing and sanitisation. All three need to be achieved in order to reduce the risk.

In 2011, the FSA released guidance documents for food business operators (retail, catering and other processing sectors) and enforcement authorities for the control of *E. coli* O157 cross contamination[25]. The documents highlight the importance of physical separation of raw and cooked foods, hand washing and disinfection procedures for the prevention of cross contamination. They also outline the role of enforcement authorities to ensure adequate procedures and controls are in place within food businesses.

The United States Department of Agriculture-Food Safety and Inspection Service (USDA-FSIS) designated *E. coli* O157:H7 as a food adulterant in 1994 and subsequently developed programs to test for its presence in beef. In 2008, USDA-FSIS officials announced the

[21] http://biocontrolsys.com/ accessed 18 June 2012
[22] http://www.foodsmart.govt.nz/food-safety/foodborne-illnesses/e-coli-0157/questions-answers.htm accessed 17 May 2012
[23] http://www.cdc.gov/nczved/divisions/dfbmd/diseases/ecoli_o157h7/index.html accessed 17 May 2012
[24] http://www.food.gov.uk/consultations/consulteng/2010/reducingriskecolio157eng accessed 17 May 2012
[25] http://www.food.gov.uk/foodindustry/guidancenotes/hygguid/ecoliguide accessed 17 May 2012

intention to begin testing foods for the presence of six additional STEC serogroups (O26, O111, O145, O103, O45 and O121). This decision was made after considering information in a Risk Profile (FSIS, 2012).

Regulatory sampling for the six non-O157 serogroups was introduced on 4 June 2012, and the USDA will take action on positive samples following the same procedures as those currently followed with respect to samples that test positive for *E. coli* O157:H7.

A systematic review and meta-analysis of published studies of interventions to control *E. coli* during primary processing has been published (Greig *et al.*, 2012). This study identified that potable water final carcass wash, steam or hot water pasteurisation, and dry chilling all effectively decreased the odds and concentration of generic *E. coli* contamination on beef carcasses. However, the review also identified that there was a lack of intervention studies where pathogenic strains of *E. coli* were measured.

The Canadian regulations on *E. coli* O157:H7 are set out in guidelines provided by Health Canada, which stipulate the procedures for the handling of *E. coli* O157:H7 positive raw ground beef and for the preparation of fermented meat products containing beef (Gill and Gill, 2010).

5.2 Additional Options for Control

As noted in Section 3.3.2, reported rates of infection in rural areas appear to be higher than for people living in urban areas. The recent case-control study identified the presence of cattle and contact with animal manure as risk factors, along with contact with recreational water. This suggests that on-farm control measures addressing shedding may offer risk management for rural dwelling people, as well as reducing contamination of livestock prior to processing.

There are three areas for potential control of STEC in red meat:

(i) Reduce the prevalence of STEC in animals grown for meat and or in the environment, and the amount of STEC in animal faeces.
(ii) Reduce the transfer of STEC and faeces between animals during transport to and housing (lairage) before slaughter.
(iii) Reduce contamination of the carcass and meat during processing post slaughter.

More effective control is likely to result from a combination of interventions across these areas. The effectiveness of processes in place post slaughter to eliminate contamination of carcasses will depend on the amount of STEC associated with the animals pre-slaughter (Arthur *et al.*, 2007; Soon *et al.*, 2011).

Bacteriophage offer a pre-slaughter control tool, particularly to reduce contamination on hides (Arthur *et al.*, 2007; Soon *et al.*, 2011). Bacteriophage control is most effective when administered immediately prior to slaughter, before re-growth of resistant strains can occur.

Other options for on-farm controls include; diet manipulation (Callaway *et al.*, 2009; Jacob *et al.*, 2008), a microcin used for competition between *E. coli* strains (Eberhart *et al.*, 2012), changes to husbandry practices which reduce cross-contamination, such as providing clean and

dry shelter (Soon *et al.*, 2011), and vaccination to control prevalence and days of supershedding (Fox *et al.*, 2008; Smith *et al.*, 2009).[26]

Potential controls at the processing plant include chemicals (including antimicrobials), and hot water washes (Carlson *et al.*, 2008; Geornaras *et al.*, 2012; Kalchayanand *et al.*, 2012; Loretz *et al.*, 2011), the use of extracts of essential oils (McDonnell *et al.*, 2012) and treatments to reduce bio-film formation on equipment surfaces (Lynnes *et al.*, 2014).

[26] For example: http://www.bioniche.com/newsroom_QandA.cfm accessed 26 July 2013.

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7 APPENDIX 1: HAZARD AND FOOD

7.1 STEC

The 2002 Risk Profile contains general information on the growth, survival and inactivation of *E. coli* O157:H7 and non-O157 STEC. Microbial datasheets on these organisms are available at the MPI website.[27]

7.2 Detection and Typing

A list of validated test kits for STEC, and their serotype/gene targets, has been published by the United States Department of Agriculture Food Safety and Inspection Service (FSIS). [28]

A number of automated testing systems have been developed, including the Atlas STEC EG2 Combo Detection Assay (Roka Biosciences, San Diego, CA, USA), NeoSEEK™ (Neogen, Lansing, MI, USA) and the Assurance GDS system29 (Biocontrol, Bellevue, WA, United States). These use multiple gene targets and DNA detection techniques.

The terms "*subtyping*" or "*typing*" describe a test or assay which is able to distinguish isolates of a microbial species from each other. Subtyping tools can be valuable for:

- Outbreak identification
- Population studies, and,
- Further characterisation of the pathogen.

Serotyping of STEC is based on the O (Ohne) antigen and the H (Hauch) antigen. There are approximately 187 O antigens which are determined by the polysaccharide portion of the cell wall lipopolysaccharide, and 56 H antigens which are determined by flagella protein. Non-motile isolates (normally recorded NM) are considered here to be H-, i.e. without an H antigen.

Serotyping of STEC isolates is routinely performed in New Zealand by the Enteric Reference Laboratory at ESR (part of the National Centre for Biosecurity and Infectious Disease) (NCBID), with all isolates also analysed using a multiplex PCR that detects the *stx1*, *stx2*, *eae*, and *hlyA* genes. Isolates are also analysed by PFGE.

New typing systems continue to be developed, including a method based on PCR binary typing system derived from the presence or absence of a set of putative virulence genes (Brandt *et al.*, 2011).

Other molecular techniques based on the analysis of multiple genetic loci have provided information on important virulence mechanisms and evolutionary relationship among various STEC strains (Coombes *et al.*, 2008; Ogura *et al.*, 2009). It is anticipated that the application of these technologies in the future will complement existing PFGE-based methods for STEC,

[27] http://www.foodsafety.govt.nz/science-risk/hazard-data-sheets/pathogen-data-sheets.htm accessed 26 July 2013
[28] http://www.fsis.usda.gov/wps/wcm/connect/f97532f4-9c28-4ecc-9aee-0e1e6cde1a89/Validated-Test-Kit-Spreadsheet.pdf?MOD=AJPERES accessed 15 October 2014
[29] http://biocontrolsys.com/ accessed 18 June 2012

leading to faster, more accurate data acquisition and increased likelihood of identifying an outbreak earlier.

7.2.1 PFGE

Restriction enzyme digestion and PFGE has been extensively developed for *E. coli* O157:H7, particularly for outbreak investigations and is a widely applied method of subtyping STEC. In this method, fragments of bacterial chromosome generated by digestion with a restriction enzyme selected to cut the DNA into 20-25 pieces are separated by electrophoresis. Fingerprint patterns (bar-code like patterns that tend to be the same among strains from a common source) are compared using a centralised database system facilitating the identification, tracing and prevention of food and waterborne disease outbreaks. The databases also assist in the identification of changes in strain distributions and the emergence of new strains. As the enzymes used and the conditions under which the gel electrophoresis is undertaken can have a marked influence on the end result, standardised protocols are essential. PFGE has made it possible for specific STEC serotypes to be linked in outbreaks and the information gathered can be compared across the PulseNet system in the US and other countries.[30] New Zealand is part of the PulseNet Asia Pacific branch through the participation of ESR.[31]

Two reports have been prepared by ESR for the New Zealand Food Safety Authority describing some PFGE typing data for *E. coli* O157:H7 isolates. The first was a response to suspicions in the US that a PFGE type causing disease in the US could have originated from New Zealand meat (Cornelius *et al.*, 2006). A subtype present in the US is also common in New Zealand when assessed by the pattern produced by one restriction enzyme (*Xba*I). However, analysis using a second enzyme showed that the New Zealand isolates were distinguishable from those in the US. Further work on these and other isolates was subsequently reported (Cornelius, 2009).

7.2.2 MLVA

Multiple locus variable number tandem repeat analysis (MLVA) is a PCR based method that detects the occurrence of tandem duplication on stretches of DNA at specific loci in the chromosome. This method is based on variations in nucleotide sequences of internal fragments of selected housekeeping genes and has not been found to be effective in finding diversity between STEC that has been found using PFGE. MLVA is currently used for *E. coli* O157:H7 in several PulseNet laboratories worldwide and a protocol is available for *E. coli* O157 and under development for non-O157[32].

7.2.3 SBI

Stx encoding bacteriophage insertion (SBI) typing uses the property that the Shiga toxin(s) (Stx) are encoded by bacteriophages inserted into the bacterial genome. These can be identified and discriminated using sequence analysis (Besser *et al.*, 2007; Shaikh and Tarr, 2003). This typing method is used as an adjunct to serotyping and PFGE typing.

[30] http://www.pulsenetinternational.org/Pages/default.aspx accessed 30 May 2012

[31] http://www.pulsenetinternational.org/networks/Pages/asiapacific.aspx accessed 30 May 2012

[32] http://www.pulsenetinternational.org/protocols/Pages/mlva.aspx accessed 30 May 2012

7.3 Exposure Assessment Tables

The following tables supplement information presented in Section 2.4.

Table 7: STEC counts and serotypes isolated from New Zealand retail uncooked meats showing carriage of virulence genes (Wong *et al.*, 2006).

Meat type	*E. coli* Serotype	Virulence factors detected by PCR				Count in MPN/g (95% CI)
		*stx*1	*stx*2	*eae*A	*hly*A	
Beef						
Minced	ONT:H26	x			x	1.0 (0.35-4.78)
Minced	ONT:HNM	x			x	<0.33 [a]
Minced	O163:H19		x		x	<0.33
Minced	O149:HNM		x			<0.33
Minced	ONT:H17	x			x	<0.33
Diced	O22:HNM		x		x	<0.33
Minced	O75:H10		x			<0.33
Minced	O75:HNM		x			<0.33
Minced	O38:H26	x			x	<0.33
Minced	O38:H26	x	x		x	ND
Minced	O113:H21		x		x	<0.33
Minced	O75:H8	x	x		x	<0.33)
Pork						
Minced	O157:[H7]		x	x	x	<3.3
Minced	O9:H10		x			<0.33
Minced	ONT:HNM	x				<0.33
Minced	ONT:H26	x			x	<0.33
Minced	ONT:HNM	x			x	1.0 (0.35-4.78)
Diced	O38:H26		x		x	<0.33
Minced	ONT:HNM		x			<0.33
Minced	ONT:HNM	x				<0.33
Diced	ONT:HNM	x			x	0.33 (0.10-2.36)
Minced	O163:H19		x		x	<0.33
Minced	O15:HNM	x			x	<0.33
Minced	O128:HNM		x		x	<0.33
Minced	ONT:HNM	x	x		x	<0.33
Minced	ONT:HNM	x			x	<0.33
Minced	O176:H4	x			x	<0.33
Unweaned veal						
Frozen shank	O26(rel):H11	x		X	x	<0.33
Frozen shank	O26(rel):H11	x		X	x	<0.33
Frozen shank	O5:HNM	x			x	<0.33
Frozen shank	O157:H7	x	x	X	x	<0.33)
Lamb/mutton						
Diced	O128:H11	x	x		x	<3.3[b]
Minced	O157:H7		x	X	x	3.3 (0.99-23.61)
Minced	O9:H10		x			<0.33
Minced	Orough:H2	x	x		x	0.33 (0.10-2.36)
Diced	O38:H26	x				>1.0
Diced	ONT:H10	x	x		x	<0.33
Mutton-diced	O178:H7	x				0.33 (0.10-2.36)

Meat type	*E. coli* Serotype	Virulence factors detected by PCR				Count in MPN/g (95% CI)
		*stx*1	*stx*2	*eae*A	*hly*A	
Diced	ONT:HNM	x			x	0.33 (0.10-2.36)
Minced	ONT:HNM	x			x	>1.0
Diced	O38:H26		x			1.0 (0.35-4.78)
Mutton-minced	O157:H7		x	X	x	<0.33
Diced	O171:H2		x			<0.33
Minced	Orough:HNM	x	x		x	<0.33
Diced	O6(rel):HNM	x	x		x	<0.33
Minced	O5:HNM	x			x	<0.33
Minced	ONT:H17	x			x	<0.33
Minced	O5:HNM	x			x	<0.33
Minced	O5:HNM	x			x	<0.33
Diced	O15:H27	x	x			<0.33
Mutton-minced	ONT:H25		x			<0.33
Minced	O38:H28	x	x		x	<0.33
Minced	O6:HNM	x	x		x	>1.0
Diced	O8:H25		x			<0.33
Minced	ONT:H17	x			x	<0.33
Minced	O68:H4	x			x	<0.33
Minced	O75:H8	x	x		x	1.0 (0.35-4.78)
Minced	O128:H2	x			x	<0.33
Diced	O157:[H7]		x	X	x	1.0 (0.35-4.78)
Minced	O123:H10	x	x		x	<0.33
Diced	O128:H2	x	x			<0.33
Minced	O176:H4	x			x	<0.33
Diced	O75:H8	x	x		x	<0.33
Minced	O176:HNM	x	x		x	<0.33
Diced	O5:HNM	x			x	<0.33

[a] <0.33 MPN/g represents a count between 1 to 8.25 MPN in 25g.
[b] <3.3 MPN/g was due to the inoculation of 1 ml of homogenate to the 3-tube MPN instead of 10 ml of inoculum per tube.
CI = Confidence interval

Table 8: International comparison of red meat consumption, 2007 (kg/person/year) (from Food Balance Sheets)[33]

Country	Meat Consumption (kg/person/year)			
	Bovine meat	Sheep and Goat meat	Pigmeat	Total
Argentina	54.9	1.4	6.7	62.6
Australia	44.0	14.5	23.3	81.8
Canada	32.8	1.2	27.4	61.4

[33] http://faostat.fao.org/site/368/DesktopDefault.aspx?PageID=368#ancor accessed 28 May 2012

Country	Meat Consumption (kg/person/year)			
Denmark	26.7	1.2	49.7	77.6
France	26.9	3.3	31.8	62.0
Germany	13.2	0.7	55.7	69.6
Greece	18.4	13.9	27.4	59.7
Italy	24.1	1.4	44.8	70.3
New Zealand	32.1	23.3	22.9	78.3
Sweden	24.0	1.2	36.4	61.6
United Kingdom	22.0	6.1	27.8	55.9
United States	41.2	0.5	29.7	71.4

Table 9: Prevalence of *Escherichia coli* O157:H7 in meat from overseas surveys

Country	Products tested	Number tested	% positive	Reference
Argentina	Beef and chicken burgers	279	6.8	(Chinen *et al.*, 2009)
Republic of Ireland	Beef trimmings Beef carcasses Head meat	1351 132 100	2.4 3.0 3.0	(Carney *et al.*, 2006)
Republic of Ireland	Fresh burgers (MAP)-supermarket Fresh mince (unpackaged)-butchers Frozen burgers (packaged)-supermarket	157 211 206	4.46 3.79 2.91	(Cagney *et al.*, 2004)
	Fresh mince (MAP)-supermarket Fresh burgers (unpackaged)-butchers Fresh mince (unpackaged)-supermarket Fresh burgers (unpackaged)-supermarket	457 140 299 63	2.84 2.14 2.01 0	
France	Minced beef	3450	0.12	(Vernozy-Rozand *et al.*, 2002)
Italy	Minced beef Mixed minced beef and chicken	75 10	0 0	(Stampi *et al.*, 2004)

Country	Products tested	Number tested	% positive	Reference
	Hamburger Hamburger with vegetables Meatballs	30 24 10	3.3 8.3 0	
Italy	Minced beef	931	0.4	(Conedera *et al.*, 2004)
Morocco	Meat and meat products	460	0.9	(Badri *et al.*, 2011)
Palestine	Raw beef	300	14.7**	(Adwan and Adwan, 2004)
USA	Minced beef	296	16.8**	(Samadpour *et al.*, 2002)
USA	Beef carcasses Beef carcasses	1232 1232	1.2 16.2**	(Barkocy-Gallagher *et al.*, 2003)

*Only three isolates from 10 presumptive positives were obtained, and only one of these three (from beef) had the full complement of pathogenicity genes and activities examined. True adjusted prevalences are therefore 0% for pork and 0.3% for beef.
** This value is for STEC
MAP=Modified atmosphere packaging

Table 10: Quantitative data for the prevalence of *Escherichia coli* O157:H7 in meat products

Country	Products tested	Concentration	Reference
Republic of Ireland	Beef trimmings Beef carcasses Head Meat	<0.70 to 1.61 $\log_{10}$ CFU/g <0.70 to 1.41 $\log_{10}$ CFU/g 0.70 to 1.0 $\log_{10}$ CFU/g	(Carney *et al.*, 2006)
Republic of Ireland	Fresh mince (unpackaged)-butcher Fresh burger (unpackaged)-butcher Fresh mince (MAP)-supermarket Fresh mince (unpackaged)-supermarket Frozen burgers (packaged)-supermarket Fresh burgers (MAP)-supermarket	E to 2.40 $\log_{10}$ CFU/g E and 0.90 $\log_{10}$ CFU/g E to 4.03 $\log_{10}$ CFU/g E to 3.43 $\log_{10}$ CFU/g E to 0.51 $\log_{10}$ CFU/g E to 3.04 $\log_{10}$ CFU/g	(Cagney *et al.*, 2004)
France	Frozen burgers	5.9 CFU/g	(Delignette-Muller *et al.*, 2008)

E = positive by enrichment only.
MAP = Modified Atmosphere Packaging

8 APPENDIX 2: EVALUATION OF ADVERSE HEALTH EFFECTS

8.1 Adverse Health Effects Overseas

8.1.1 Serotypes causing disease

Overseas, the number of reported cases of non-O157 STEC infection appear to be increasing, but this is not necessarily due to increasing infections; recognition of non-O157 STEC infection as a notifiable disease and increased laboratory testing for these organisms in diarrheal patients means that non-O157 STEC infection is now more likely to be reported (Mathusa *et al.*, 2010). In the United States, the number of reported non-O157 STEC infections increased from an incidence of 0.12 per 100,000 population in 2000 to 0.95 per 100,000 in 2010; while the rate of O157 STEC infections decreased from 2.17 to 0.95 per 100,000 (Gould *et al.*, 2013). In Australia the rate of STEC infection is higher for South Australia because laboratories in this State routinely test all bloody stools by PCR for the *stx* genes as part of diagnosis (OzFoodNet, 2012). Data presented in Table 11 show the proportion of serotyped isolates that are non-O157 STEC, which varies between countries. It is beyond the scope of this Risk Profile to investigate the extent to which these differences are a result of different laboratory testing protocols.

A review of non-O157 serotypes, their role in causing disease, and the association with beef, found that very few public health laboratories in Canada conduct tests for non-O157 STEC and that relatively few cases of non-O157 STEC illness are reported because of the lack of systematic testing for these organisms (Gill and Gill, 2010). However, the limited data available suggested that in Canadian beef the prevalence of non-O157 serotypes was equal to, and possibly greater than the prevalence of *E. coli* O157.

In Argentina 59% of isolates from symptomatic children were *E. coli* O157:H7, with the next most frequently isolated serotypes being O145:NM, O26:H11, O113:H21 and O174:H21 (Rivas *et al.*, 2003).

A study of isolates from patients in Germany indicated that while *E. coli* O157 was the single most frequently occurring serotype it only accounted for 25% of the isolates, with O26 (12.9%), O103:H2 (5.6%), O91 (5.5%), O111 (4.1%), O145 (3.5%), O128:H2 (3.4%), O113 (2.5%), O118:H16 (2.2%), O76 (2.2%) and O146:H21 (2.2%) predominant among the other isolates (Beutin *et al.*, 2004).

8.1.2 Classification of STEC for pathogenicity

The review by EFSA proposed establishing a seropathotype group: "haemolytic uraemic syndrome (HUS)-associated serotype(s)" (EFSA, 2013). In the EFSA report, as of 2013, this group includes: HAS = HUS-associated serotypes (that have been associated with reported confirmed HUS cases of human VTEC in EU in 2007-2010): O157:H7, O157:H-, O121:H19, O26:H11, O174:H2, O111:H-, O145:H-, O145:H28, O1:H42, O128:H2, O111:H8, O104:H21, O174:H21, O7:H6, O76:H19, O80:H2, O86:H27, O121:H2, O123:H2, O105:H18, O91:H10.

In terms of genetic elements associated with virulence, EFSA stated:

"The detection of verocytotoxins alone or genes encoding for such verocytotoxins is not a sound scientific basis for assessing the disease risk to the consumer.

There is no single or combination of marker(s) that defines a "pathogenic" VTEC. Strains positive for verocytotoxin 2 gene (*vtx2*)- and *eae* (intimin production)- or [*aaiC* (secreted protein of EAEC) plus *aggR* (plasmid-encoded regulator)] genes are associated with a higher risk of more severe illness than other virulence factor combinations. Other virulence gene combinations and/or serotypes may also be associated with severe disease in humans, including HUS.

A molecular approach, utilising genes encoding virulence characteristics additional to the presence of *vtx* genes, is proposed. This molecular approach must be regarded as provisional because screening VTEC for the presence of *eae*, *aaiC* or *aggR* genes is not routinely undertaken. This scheme has the advantage of overcoming problems associated with the lack of flagella "H" antigen typing. The performance of this proposed approach needs to be verified with well-characterised isolates from cases of human infection and from food-producing animals and foods."

The EFSA report further stated:

"Pathogenicity can neither be excluded nor confirmed for a given VTEC serogroup or serotype based on the seropathotype concept or analysis of the public health surveillance data.

On the basis of the proposed molecular classification scheme, any RTE product contaminated with an isolate of one of the VTEC serogroups of group I (O157, O26, O103, O145, O111, O104) in combination with *vtx* and [1] *eae* or [2] *aaiC* and *aggR* genes should be considered as presenting a potentially high risk for diarrhoea and HUS. For any other serogroups in combination with the same genes, the potential risk is regarded as high for diarrhoea, but currently unknown for HUS. In the absence of these genes, current available data do not allow any inference regarding potential risks."

8.1.3 Incidence

The 2002 Risk Profile presented incidence data for STEC infection for Australia, the USA and some European countries. More recent incidence data are given in Table 11, with New Zealand data provided for comparative purposes. The rate per 100,000 is increasing for most of these countries, as is the proportion of isolates serotyped as being non-O157.

Table 11: Rates of reported STEC infections by country

Country	Year		Serotyped isolates	Ref.*

		Incidence (per 100,000)	Number	% O157	% other STEC	
New Zealand	2012	3.3	142	84	16	a
	2013	4.6	215	89	11	b
Australia	2011	0.4	NR	NR	NR	c
	2010	0.4	51	59	41	d
European countries						
EU[1]	2012	1.1	3482	56	44	e
Austria	2012	1.5	109	16	84	
Belgium	2012	0.9	105	62	38	
Czech Republic	2012	0.1	9	56	44	
Denmark	2012	3.5	178	21	79	
France	2012	0.3	205	33	77	
Germany	2012	1.9	471	18	82	
Ireland	2012	9.0	393	48	52	
Italy	2012	0.1	43	33	67	
Netherlands	2012	6.3	286	31	69	
Spain	2012	<0.1	31	77	23	
Sweden	2012	5.0	292	24	76	
United Kingdom	2012	2.2	3482	97	3	
North American countries						
Canada	2011	1.4[2]	527	91	9	f
USA (O157)[3]	2012	1.1	N/A	N/A	N/A	g
USA (nonO157)[3]	2012	1.2	N/A	N/A	N/A	g

NR, Not Reported

Table notes:

1. EU values based on data from 26 Member States.
2. Rate is for O157 STEC only. Rate for non-O157 STEC has been in the range 0.12 to 0.41 per 100,000 over a ten-year period (2001-2011; NESP 2013, page 16).
3. Data is for the 10 sentinel states monitored by FoodNet, not the whole of the USA.

** References:*

a. (Lopez *et al.*, 2013)
b. (Horn *et al.*, 2014)
c. (Department of Health and Aging, 2013)
d. (OzFoodNet, 2012)
e. (European Food Safety Authority and European Centre for Disease Prevention and Control, 2014)
f. (NESP, 2013)
g. (CDC, 2013)

8.1.3.1 Community level estimates

The number of notified STEC infections only represents a proportion of total cases, as not all cases will come into contact with public health agencies.

In Australia, an estimate for the number of annual community STEC infections was 4,420 (95% Credible Interval (CrI): 2,407-10,196) (Hall *et al.*, 2008). This was based on notified cases from 2000 through 2004, and corresponded to an estimated rate of 23 (95% CrI: 13-54) cases of STEC infection per 100,000 people, per year.

In the USA, surveillance data from 2000 to 2008 were used to calculate estimates of the overall number of domestically-acquired episodes of illness caused by STEC infection (Scallan *et al.*, 2011). The estimated mean number of O157 STEC cases was 93,094 (90% CrI: 26,046-219,676). The estimated mean number of non-O157 STEC cases was 138,063 (90% CrI: 14,080-350,891). Applying the 2006 USA population of 299 million (as used by Scallan *et al.* 2011) to the mean values, this corresponds to rates per 100,000 of 31 for O157 STEC infection and 46 for non-O157 STEC infection. Estimates were also made for the number of domestically-acquired foodborne infections; using the same approach, these means convert to rates of 21 cases of O157 STEC infection per 100,000 and 38 cases of non-O157 STEC infection per 100,000.

Estimates of domestically-acquired foodborne illness cases have been published for Canada (Thomas *et al.*, 2013). The estimates were based on surveillance data from 2000 to 2010 plus relevant international literature, and were produced through a modelling approach that accounted for underreporting and underdiagnosis. The researchers estimated 39 cases of O157 STEC infection per 100,000 people per year and 63 cases of non-O157 STEC infection per 100,000 people per year.

The community rate of infectious intestinal disease caused by *E. coli* O157 found in the prospective IID2 study in the United Kingdom was 30 per 100,000 person years (Tam *et al.*, 2012).

8.1.4 Contributions to Outbreaks and Incidents

The 2002 Risk Profile listed 31 outbreaks of STEC infection associated with meat products. Between 1982 and 2002, 41% of outbreaks in the US involved minced beef as the vehicle with a further 6% attributed to "other beef" (Rangel *et al.*, 2005). More recent examples of reported incidents of disease associated with meat products are listed in Table 12 and Table 13.

Table 12: Examples of specific incidents of disease reported for *Escherichia coli* O157:H7 associated with meat products

Location	Setting	No. affected	No. deaths	Source	Reference
Argentina	Home	1	0	Hamburger	(Rivas *et al.*, 2003)
Canada	Community	143 (6 HUS)	0	Salami	(MacDonald *et al.*, 2004)
Canada	Community	9	0	Beef donair	(Honish *et al.*, 2007)
France	Community	26 (13 HUS)	0	Beef burgers	(French multi-agency outbreak investigation team, 2005)
Japan	Community	28	NS	Restaurant beef	(Tsuji *et al.*, 2002)
Netherlands	Community	20	0	Steak tartare	(Greenland *et al.*, 2009)
Scotland	Community	9	1	Cooked meat	(Stirling *et al.*, 2007)
USA	Community	46	NS	Minced beef	(Gerner-Smidt *et al.*, 2005)

Location	Setting	No. affected	No. deaths	Source	Reference
USA	Community	64	NS	Minced beef	(Gerner-Smidt *et al.*, 2005)
USA	University	61	0	Roast Beef	(Rodrigue *et al.*, 1995)
USA	Home	1	0	Deer meat	(Rabatsky-Ehr *et al.*, 2002)
USA	Community	10 (1 HUS)	0	Nonintact steaks	(Laine *et al.*, 2005)
USA	Community	45 (1 HUS)	0	Minced beef	(Centers for Disease Control, 2008)
Wales	Community	157	1	Cooked sliced meats	(Pennington, 2009)

NS=Not Stated

Table 13: Specific Incidents of Disease Reported for non-O157 STEC Associated with Meat Products

Serotype	Location	Setting	No. affected	No. deaths	Source	Reference
O26:H11	Germany	Community	11 (5 asymptomatic)	0	Beef product- "Seemerolle"	(Werber *et al.*, 2002)
O26:H11	Denmark	Community	20, 1 case had bloody diarrhoea	0	Beef sausage	(Ethelberg *et al.*, 2009)
O103:H25	Norway	Community	17 (10 HUS)	1	Mutton sausages	(Schimmer *et al.*, 2008)

HUS = Haemolytic Uraemic Syndrome, GI = Gastrointestinal

8.1.5 Case control studies

The 2002 Risk Profile listed six overseas case control studies investigating consumption of meat products as risk or protective factors. Studies published since 2002 are summarised in Table 14.

Table 14: Case control studies where meat consumption was identified as a risk factor for infection with STEC

Location	Risk factors identified	Reference
Argentina	Consumption of undercooked beef (sporadic cases)	(Rivas *et al.*, 2008)
Australia	Eating hamburgers	(McPherson *et al.*, 2008)
Germany	Lamb and spreadable sausage in cases >10 years of age (sporadic cases)	(Werber *et al.*, 2007)
USA	Eating pink hamburgers	(Voetsch *et al.*, 2007)
USA	Eating undercooked hamburgers (sporadic cases)	((Kassenborg *et al.*, 2004)

8.2 Risk Assessments and Other Activities Overseas

A review has been undertaken of the quantitative microbial risk assessments for *E. coli* O157 in beef up to 2006 (Duffy *et al.*, 2006b). It was concluded that all of the models could be adapted to use input data such as prevalence and concentration on carcasses for a particular region. The models also differ in their dose response parameters and this greatly influenced the risk, but none of the three principle dose response models were considered to be ideal. Four of the five models indicated that the biggest influence on risk was the concentration of STEC in the faeces or on the hide of the animal presented for slaughter. Temperature of storage at retail was also an important factor in determining risk.

A quantitative microbial risk assessment for *E. coli* O157:H7 in beefburgers produced in the Republic of Ireland was published in 2006 with the slaughter module of the assessment published as a paper in 2008 (Cummins *et al.*, 2008; Duffy *et al.*, 2006a). The study examines contamination of beef trimmings in Irish abattoirs including initial contamination levels, cross-contamination and decontamination events during the cattle slaughter process. The mean simulated prevalence of *E. coli* O157:H7 on trimmings was 2.36% and the mean simulated counts of *E. coli* O157:H7 on contaminated trimmings was -2.69 $\log_{10}$ CFU/g. The retail/domestic part of the model was based on studies of consumer storage and cooking practices, retail storage conditions. Beef burgers made from beef trim were assumed to be cooked well done by 87% of consumers, medium by 12% and 1% rare. Using an exposure assessment and a dose-response relationship the model predicted that the risk of human illness from the consumption of a serving of minced beef and beefburgers was 1.1×10^{-6}, which is approximately one illness per million burgers consumed (Powell *et al.*, 2000).

An Argentinian quantitative risk assessment for STEC (all serotypes) covered the food chain from the prevalence on farm, through primary processing, commercial and home preparation of hamburger patties, and domestic cooking and consumption (Signorini and Tarabla, 2009). Exposure estimations were applied to a dose-response relationship to estimate the probability of illness, HUS and mortality. These were an infection risk of 8.12×10^{-7}, a probability of HUS of 4.6×10^{-8} and probability of mortality of 5.9×10^{-9} per meal for adults. The risk of infection was sensitive to the type of storage at home, slaughterhouse storage temperature and bacterial concentration on the cattle hide. The risk of illness was highly correlated with the initial concentration of the pathogen in cattle. Therefore, it was recommended that risk management should focus on reducing the microbial load in the raw material. Previous studies had demonstrated that the prevalence of *E. coli* O157:H7 in cattle faeces varied from 0.1% to 53% (Duffy *et al.*, 2006a). In the Argentinian study, the average prevalence of pathogenic strains of *E. coli* STEC was 21%. The absence of local information on the *E. coli* VTEC counts on bovine hides and carcasses was recognised as one of the most important limitations of this model. The most important factor associated with the risk for infection was the pathogen concentration on the hides of cattle at slaughter, followed by temperature during meat processing, and thermal abuse during storage and food preparation.

An attribution study of human STEC O157 infection from meat products in the UK was undertaken using a quantitative risk assessment approach (Kosmider *et al.*, 2010). This study used a stochastic model to simulate the prevalence and amount of STEC O157 in pork, lamb and beef products and concluded that the prevalence of STEC O157 in meat products (joints and mince) at consumption was low (i.e. <0.04%). Beef products, particularly beef burgers, presented the highest estimated risk with an estimated eight out of 100,000 servings on average resulting in human infection with STEC O157.

The FSIS/USDA has undertaken a farm to table risk assessment for *E. coli* O157:H7 in ground beef (preliminary results were reported in the 2002 Risk Profile).[34] The risk assessment was intended to identify the occurrence and concentration of this pathogen at specific points, to contribute to a risk reduction strategy and to identify future research needs. Results from this risk assessment indicate that the median annual risk of *E. coli* O157:H7 infection from ground beef for the general population is nearly 1 illness in 1 million servings of ground beef, with the corresponding risk of death at 5.9×10^{-10} per serving. While the risk assessment contains a significant amount of useful reference material the study is very much oriented to the American meat production system, as would be expected. Since the New Zealand situation is very different with, for example, the virtual absence of feedlot cattle and a different seasonal pattern of human infections, little can be taken from the risk assessment that would assist with the New Zealand situation. The document does list several data gaps, and carries out sensitivity analysis on factors that could help to mitigate disease. Interestingly the effect of reducing the proportion of contaminated lots of beef seemed to be greater than reducing the level of contamination. Other conclusions, such as the projection that adequate storage of minced beef and correct cooking of hamburgers, meat balls, meat loaf etc. would result in a huge decrease in cases seem obvious. However, given that the transmission routes in New Zealand are unclear, the effect of these interventions cannot be extrapolated to New Zealand.

The FSIS/USDA has also produce a risk assessment comparing the risk for intact and non-intact beef.[35] It was concluded that the probability of one or more cells of the pathogen surviving typical cooking practices was "miniscule" (of the order of 3 per 10 million servings) for both intact and non-intact steaks. However, as demonstrated by the outbreak cause by non-intact steak consumption (Laine *et al.*, 2005), the risk of the non-intact steaks lies in their being undercooked.

A risk assessment for *E. coli* O157:H7 in raw beef patties has been produced for the Netherlands (Nauta *et al.*, 2001). The simulation predicted that 0.3% of patties would contain at least 1 cell, whereas a small survey (82 samples) detected the organism in 1.2%. The concentration of the pathogen in the patties was predicted to be low with 60% of the contaminated patties containing only 1 CFU and only 10% contained more than 10 CFU. It was concluded that farm-level interventions are likely to be more effective at reducing disease than those targeted at the consumer.

The most recent model located focused on an assessment of the risk posed by beef patties in France (Delignette-Muller *et al.*, 2008). This used data from a single outbreak and focused on the risk to children in the 0-5 and 5-10 years old brackets. A dose response model was developed for HUS in people in these two age groups (see dose response section). Cooking of patties was an important factor for risk, and a smaller proportion of French consumers eat hamburgers well-cooked than do people in other countries.

A risk assessment of *E. coli* O157 illness in the USA due to consumption of 155 tonnes of Australian beef estimated that 50 illnesses would occur (Kiermeier *et al.*, 2014). This risk assessment model was based on the prevalence and concentration estimates of *E. coli* O157 in beef lots withdrawn following routine testing. The final risk assessment was based on the assumption that no lots were withdrawn, and is likely therefore to overestimate the number of illnesses.

[34] http://www.fsis.usda.gov/OPPDE/rdad/FRPubs/00-023N/00-023NReport.pdf accessed 29 May 2012
[35] http://www.fsis.usda.gov/PDF/Beef_Risk_Assess_Report_Mar2002.pdf accessed 29 May 2012