



Microbiological safety of raw minced beef and beef burgers on retail sale in Ireland (11NS1)

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SUMMARY

This survey investigated the prevalence of *Salmonella* and verotoxigenic *Escherichia coli* (VTEC) in samples of raw minced beef and raw beef burgers from retail outlets and catering premises in Ireland. *Salmonella* (identified as *Salmonella* Dublin) was detected in 0.1% of samples (1/983). *E. coli* O157 was detected in 0.2% (2/983) of samples specifically tested for this serogroup while VTEC were detected in 2.5% (10/402) of samples when tested using a non-serogroup specific polymerase chain reaction (PCR) test for VTEC. In all, eight VTEC serogroups were detected: O6 (x4), O157 (x2), O8, O130, O145, O149, O166 and a VTEC O-unidentifiable.

Based on information provided by the survey questionnaires, 12% (85/731) of samples were found to be stored or displayed in the retail or catering establishment at a temperature higher than the recommended temperature of 5°C or less. These included three samples in which VTEC were detected.

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INTRODUCTION

This survey investigated the prevalence of *Salmonella* and verotoxigenic *Escherichia coli* (VTEC) in raw minced beef and beef burgers collected from catering and retail establishments in Ireland.

VTEC¹ and *Salmonella* can cause serious illness in humans. Either infection can be caused by consuming contaminated food or water, by contact with an infected person or by contact with animals or their surroundings. In Ireland, 356 cases of *Salmonella* infection and 196 cases of VTEC infection were reported to the Health Protection Surveillance Centre in 2010. In total, 38% (6/16) of the salmonellosis outbreaks, but none of the 45 VTEC outbreaks reported to the HPSC in 2010 were suspected to be caused by contaminated food (HPSC 2011).

Eating raw or undercooked minced beef or beef burgers has been reported as the cause of various outbreaks of salmonellosis and VTEC infection in Europe (Haeghebaert *et al* 2001; Isakbaeva *et al* 2005; Doorduyn *et al* 2006; Greenland *et al* 2009; Whelan *et al* 2010; Soborg *et al* 2013). *E. coli* O157, the most commonly reported strain of VTEC, was first recognised as an important pathogen in 1993, following a large outbreak in the United States linked to eating undercooked beef burgers sold from a fast-food restaurant chain. More than 700 people fell ill and four children died (Rangel 2005). Recently, an investigation was carried out into a multistate outbreak of *Salmonella* Typhimurium infection in the United States. Initial investigations focused on a group of people who ate raw ground beef kibbeh (a dish typically made of finely ground red meat, usually beef, minced onions, and bulghur wheat) at the same restaurant before becoming ill (CDC 2013).

VTEC and *Salmonella* may be found in the gastrointestinal tract of cattle and are shed in faeces (Duffy 2003; McEvoy *et al* 2003; Madden *et al* 2007). Hence, beef carcasses can become contaminated with these pathogens when faecal material from the hide or gastrointestinal tract is transferred to the carcass during hide removal and evisceration (FSAI 2010). After the slaughtering process, the surfaces of raw meat can be contaminated with VTEC and *Salmonella* as well as other pathogens such as *Campylobacter*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Clostridium perfringens* (Tompkin *et al* 2001). The microbial contamination usually occurs on the exposed surface of whole cuts of meat, but when meat is minced, microorganisms that are on the surface of the meat become mixed throughout. This is why minced meat and beef burgers should be cooked thoroughly, but steaks or whole joints of beef may be cooked rare. Cooking minced beef and beef burgers to a core temperature of 75°C or equivalent (e.g. 70°C for two minutes) is recommended (NSAI 2007a; ACMSF 2007).

Various control measures along the food chain contribute to reducing the risk of infection from minced beef and beef burgers (FSAI 2010). These include:

- Only accepting suitably clean cattle for slaughter
- Applying food safety management systems based on HACCP (Hazard Analysis and Critical Control Points) at slaughter, cutting and boning, distribution, retail and catering levels
- Maintaining chilled storage for carcasses, beef cuts, minced beef and beef burgers
- Avoiding cross-contamination between raw and ready-to-eat foods
- Fully cooking minced beef and beef burgers to a core temperature of 75°C, or an equivalent time/temperature combination

¹ VTEC is also referred to as shiga toxin producing *E. coli* (STEC)

OBJECTIVES

1. To collate baseline information on the prevalence and types of VTEC and *Salmonella* spp. in raw minced beef and raw beef burgers, intended to be eaten cooked, from retail and catering establishments in Ireland.
2. To investigate if the prevalence of VTEC has changed since the FSAI commissioned surveys in 2002 (Cagney *et al* 2004) and 2004 (Murphy *et al* 2005).
3. To investigate compliance with the microbiological limit specified in Commission Regulation (EC) No 2073/2005 (as amended) for *Salmonella* spp. in 'Minced meat and meat preparations made from other species than poultry intended to be eaten cooked'.



METHOD

Sample Collection

From April to July 2011, inclusive, environmental health officers (EHOs) collected samples of raw minced beef and raw beef burgers, which were intended to be cooked before eating, from retail and catering establishments in Ireland. EHOs only collected samples from catering establishments after the caterer confirmed that the beef would be cooked before consumption. In retail outlets, EHOs only collected samples if instructions to cook were provided on the pack or in the display cabinet in order to inform the consumer that the beef must be cooked before it is eaten.

Sample Analysis

Microbiological analysis was carried out in three official food microbiology laboratories of the Health Service Executive: Dublin Public Health Laboratory, Limerick Public Health Laboratory and Waterford Public Health Laboratory. See Appendices 1-5 for a summary of the detection methods.

Salmonella was detected using the EN/ISO 6579 method². Confirmation by serotyping was carried out at the National Salmonella, Shigella and Listeria Reference Laboratory (NSSLRL) at the National University of Ireland in Galway.

Testing for VTEC is more complicated. At the time the survey was conducted, a standardised method was only available to detect one type of VTEC, i.e. *E. coli* O157. Therefore, four methods were used to detect VTEC in this survey (see Appendix 1 for a summary):

1. A **non-serogroup specific method** for detecting VTEC. PCR was used to check for the presence of the *E. coli* genes for verotoxin 1 and/or verotoxin 2 in a sample. PCR-positive samples were then cultured and the serogroup of the resulting isolates was determined using serogroup-specific antibodies
2. ***E. coli* O157** was detected using the ISO 16654:2001 method, in which magnetic beads coated in O157 antibodies are used to capture *E. coli* O157 cells from a sample. Isolates were confirmed by serology. (Dublin Public Health Laboratory also used PCR for detection of verotoxin 1 and verotoxin 2 genes, with confirmation by serogroup gene if required.)
3. ***E. coli* O26** was detected using a modified version of the ISO 16654:2001 method, where magnetic beads coated in O26 antibodies were used to capture *E. coli* O26 cells from a sample. Isolates were confirmed by serology. (Dublin Public Health Laboratory also used PCR for detection of verotoxin 1 and verotoxin 2 genes, with confirmation by serogroup gene if required.)
4. Finally ***E. coli* O103** was detected using a PCR method specific to *E. coli* O103. Samples which were positive for *E. coli* O103 by PCR were then cultured and confirmed using O103-specific antibodies

² Dublin Public Health Laboratory used VIDAS for initial testing with confirmations in accordance with EN/ISO 6579

Result Categorisation

Results were classified according to Table 1.

Table 1: Classification of results

Microorganism	Satisfactory	Unsatisfactory
<i>Salmonella</i>	Absence in 10 g*	Presence in 10 g
VTEC (any serogroup)	Absence in 25 g	Presence in 25 g

* Legal criterion. See Commission Regulation (EC) No 2073/2005, Food Category 1.6 'minced meat and meat preparations from species other than poultry intended to be eaten cooked'.

Statistical Analysis

Fisher's Exact Test analysis was performed using SPSS version 18.0. Significance was defined at the $P < 0.05$ level.

RESULTS AND DISCUSSION

In total, 983 samples of minced beef (n=419) and beef burgers (n=564) were collected for this survey. Tests were carried for *Salmonella*, VTEC, *E. coli* O157, *E. coli* O26 and *E. coli* O103. The results are displayed in Table 2, with details of the 13 positive samples displayed in Table 3.

Table 2: Overall microbiological results

Test	Number of samples tested	Number positive	% positive
<i>Salmonella</i>	983	1	0.1
VTEC	402 ^a	10	2.5
<i>E. coli</i> O157	983	2	0.2
<i>E. coli</i> O26	981 ^b	0	0
<i>E. coli</i> O103	402 ^a	0	0

^a The 'VTEC' and '*E. coli* O103' tests were only performed on samples analysed by Dublin Public Health Laboratory (402 of the 983 samples collected)

^b The *E. coli* O26 test was not performed on two of the 983 samples collected

Table 3: Details of samples in which *Salmonella* or VTEC were detected (n=13)

Detected	Verotoxin (VT) gene	PFGE profile	Mince or burger	Temperature of storage or display (°C)	Fresh or frozen	Catering or retail establishment	Month sampled
<i>Salmonella</i> Dublin	-	-	Mince	Not stated	Fresh	Catering	June
<i>E. coli</i> O6	VT1	IE-O6-003	Mince	4	Fresh	Catering	June
<i>E. coli</i> O6	VT1	Not available	Mince	4.5	Fresh	Retail	June
<i>E. coli</i> O6	VT1	IE-O6-004	Mince	5.1	Fresh	Retail	July
<i>E. coli</i> O6	VT1	IE-O6-002	Beef burger	1.8	Fresh	Retail	June
<i>E. coli</i> O8	VT2	IE-O8-002	Mince	3.8	Fresh	Retail	April
<i>E. coli</i> O130	VT2	IE-O130-001	Mince	11	Fresh	Catering	June
<i>E. coli</i> O145	VT2	IE-O145-003	Mince	0	Not stated	Retail	August
<i>E. coli</i> O149	VT1	IE-O149-001	Beef burger	7.4	Fresh	Retail	April
<i>E. coli</i> O157	VT2	IE-O157-062	Mince	4.6	Fresh	Catering	May
<i>E. coli</i> O157	VT2	IE-O157-070	Beef burger	-18	Frozen	Catering	May
<i>E. coli</i> O166	VT1 & VT2	IE-O166-001	Beef burger	2.9	Fresh	Retail	May
<i>E. coli</i> O-unidentifiable	VT1 & VT2	Not available	Beef burger	2.3	Fresh	Catering	April

Salmonella

Commission Regulation (EC) No 2073/2005 requires absence of *Salmonella* in 10g of minced beef or beef burgers intended to be eaten cooked³ (European Commission 2005). If *Salmonella* spp. are detected, the affected batch must be withdrawn or recalled from the market in accordance with Article 19 of Commission Regulation (EC) No 178/2002 (European Commission 2002).

Salmonella (identified as *Salmonella* Dublin) was detected in one of the 983 samples tested (0.1 %). This was a sample of minced beef collected from a catering establishment. As this sample did not comply with the legal criterion, the affected batch was withdrawn from trade by the food business operator as required under Article 7 of Commission Regulation (EC) No 2073/2005.

The *Salmonella*-positive rate in this survey (0.1%) compares favourably with the European Union's average in 2010, where 2.8% of single samples² were positive for *Salmonella* (EFSA & ECDC 2012). Past surveys have detected *Salmonella* in 5.5% (5/90) of samples of minced beef collected from retail outlets in Ireland (Dempster *et al* 1986) and 1.4% (21/1,492) samples of minced beef collected in slaughterhouses and at retail level in the UK (Turnbull & Rose 1982).

³ Food category 1.6 'Minced meat and meat preparations made from other species than poultry intended to be eaten cooked' in Commission Regulation (EC) No 2073/2005. Note there is a stricter criterion of absence in 25 g for 'minced meat and meat preparations intended to be eaten raw' (Food category 1.4)

While other Irish surveys did not detect *Salmonella* in any of the 22 minced beef samples collected from retail outlets in the Dublin area (Duffy *et al* 1999), or in any of the 605 minced beef and beef burger samples tested in the Ireland's former Southern Health Board⁴ (Pierse & Connolly 1988).

Salmonella Dublin is not a common cause of human illness in Ireland, with only two cases reported in 2011 (NSSLRL 2012). However, it is the most frequently detected serogroup in bovine samples. In 2011, 95% (648/680) of *Salmonella* isolated from bovine samples in Ireland were *Salmonella* Dublin (DAFM 2012).

VTEC

Four different tests for VTEC were carried out:

1. 10/402 (2.5%) samples tested using a non-serogroup specific PCR test for VTEC were positive
2. 2/983 (0.2%) samples tested specifically for *E. coli* O157 were positive
3. 0/981 samples tested specifically for *E. coli* O26 were positive, and
4. 0/402 samples tested specifically for *E. coli* O103 were positive

Because there is no microbiological criterion in Commission Regulation (EC) No 2073/2005 for VTEC in minced beef/beef burgers, this legislation does not provide a basis on which to require a withdrawal or recall when VTEC was detected in a sample. Although Regulation (EC) No 178/2002 does require that unsafe food is withdrawn or recalled from the market, Article 14 of this Regulation states that consideration must be given to the normal conditions of use of the food by the consumer in determining if a food is unsafe. As all samples collected for this survey were raw and were intended to be cooked before consumption, a recall/withdrawal of the VTEC-positive batches was not required⁵. However, on each of the 12 occasions VTEC were detected, the food business operator was notified by the EHO and follow-up action was taken, such as reviewing hygiene practices or taking repeat samples.

The current survey shows a lower prevalence of *E. coli* O157 (0.2%) than a previous survey commissioned by the FSAI in 2001/2002, where *E. coli* O157 was detected in 2.8% (43/1,533) of raw minced beef and beef burger samples on retail sale in Ireland (Cagney *et al* 2004).

With regards to non-O157 VTEC, a FSAI-commissioned survey for *E. coli* O26 and *E. coli* O111 in retail minced beef samples, detected *E. coli* O26 in 0.25% (2/800) of samples but not *E. coli* O111 (Murphy *et al* 2005). In the current survey, no *E. coli* O26 or *E. coli* O111 was detected.

However, the current survey also tested 402 samples using a non-serogroup specific PCR test for VTEC, of which 10 samples were found to be VTEC-positive. When testing food samples for VTEC, most analyses only use the test specific to *E. coli* O157 – but this test will not pick up any non-O157 VTEC that may be present in the food sample. It's important to note that the majority (83%; 10/12) of VTEC detected in this survey were a serogroup other than *E. coli* O157. If food business operators or official agencies consider it appropriate to test raw minced beef or beef burgers for VTEC, regard should be given to testing for a range of serogroups, in particular those most frequently linked to human illness: O157; O26, O103, O91, O145 and O111 (EFSA 2007). The International Organization for Standardisation (ISO) published a standard method for the detection of five of these serogroups in 2012 (ISO 2012).

⁴ Now known as Health Service Executive Southern Region

⁵ If the minced beef or beef burgers were intended to be *eaten raw*, the presence of VTEC would not be tolerated. The affected batch would be regarded as unsafe and would be withdrawn or recalled under Article 19 of Regulation (EC) No 178/2002

In 2010, twelve EU Member States reported testing 8,566⁶ bovine meat samples for VTEC, of which 0.5% were VTEC-positive and 0.1% were VTEC O157-positive (EFSA & ECDC 2012). France reported 0.2% (6/2,476) samples of minced meat intended to be eaten cooked as VTEC positive. The serogroups detected were: O157, O26 and O145. However, Hungary and the Netherlands did not detect any VTEC in samples of minced meat intended to be eaten cooked. But these studies only tested a small number of samples: 77 and 78 samples respectively.

Recent surveys have detected a wide range of VTEC serogroups on beef farms, on bovine hides and carcasses (Byrne *et al* 2009; Ennis *et al* 2012; Monaghan *et al* 2012; Thomas *et al* 2012). Ennis *et al* (2012) isolated 33 VTEC serogroups from 12 beef farms in the north-east of Ireland. *E. coli* O157 was the only common serogroup between that survey and the current survey. Monaghan *et al* (2012) isolated 12 VTEC serogroups from bovine hides and carcasses in an Irish slaughterhouse, and the serogroups detected were different to those isolated in the current survey. Thomas *et al* (2012) tracked VTEC O157, O26, O111, O103 and O145 in Irish cattle from farm to slaughter. Two serogroups tracked were common to the current survey, i.e. O157 and O145. *E. coli* O157 was isolated from 18.9% of hides, 2.7% of faeces, 0.7% of pre-evisceration carcasses, 0.7% of post-wash carcasses, and 3.8% of environmental samples. *E. coli* O145 was isolated from 2.7% of hides, 0.7% of faeces, 0.7% of pre-evisceration and 0% of post-wash carcasses, and 1.6% of environmental samples. Byrne *et al* (2009) found 17 different non-O157 serogroups on Irish cattle farms – either *E. coli* O6 or *E. coli* O145 were present in 1% of samples tested.

Pathogenic potential of VTEC serogroups detected

VTEC strains can produce two forms of verotoxin, either VT1, VT2, or both together. Strains that produce VT2 are associated with an increased risk of haemolytic uremic syndrome (HUS), a complication of VTEC infection (HPSC 2005). Fifty-eight percent (7/12) of VTEC isolated in this survey carried the VT2 gene.

In all, eight VTEC serogroups were detected in the current survey: O6 (x4), O8, O130, O145, O149, O157 (x2), O166 and O-unidentifiable. The most common VTEC serogroups detected were O6 (33%) and O157 (17%). Although *E. coli* O157 remains the most common VTEC associated with human illness, other serogroups commonly linked to human illness in Europe include: O26, O103, O145, O111 and O91 (EFSA, 2007, FSAI, 2010). According to the Health Protection Surveillance Centre (HPSC), *E. coli* O157 and *E. coli* O145 are the only two serogroups detected in this survey which have been reported to have caused illness in Ireland. However, two asymptomatic cases of O6 VT1 and one asymptomatic case of O8 VT2 have been reported following the investigation of outbreaks with other VTEC. *E. coli* O26, the second most frequent cause of VTEC infection in humans in Ireland, was not detected in this survey.

PFGE analysis showed that all 12 VTEC isolates were unrelated. According to the VTEC Reference Laboratory at Dublin Public Health Laboratory, the patterns of both *E. coli* O157 isolates detected in this survey were indistinguishable from those of clinical isolates previously identified in Ireland.

⁶ Investigations of >25 samples

Analysis of Questionnaires

At the time of sampling, EHOs were requested to complete a survey questionnaire in order to provide more detailed information on the sample collected. The questionnaire return rate was 77% (753/983). This section of the report describes analysis of the 753 samples for which a questionnaire was returned.

The questionnaire gathered information on the type of beef (mince or burger), its state when sampled (fresh or frozen, pre-packaged or loose), where the mince or burgers were prepared (on or off premises), the type of establishment the beef was collected from (retail or catering) and the temperature of display or storage. Statistical analysis did not show that any of these variables had a statistically significant effect on the microbiological quality of the sample (Table 4).

Table 4: Beef samples for which a questionnaire was returned -- breakdown by sample detail and microbiological results

Variable		Was <i>Salmonella</i> or VTEC detected?		Statistically significant difference?*
		% No (Number negative samples / number of samples tested)	% Yes (Number of positive samples / number of samples tested)	
Type of beef	Minced beef	98.7 (305/309)	1.3 (4/309)	No (p=1.0)
	Beef burger	98.9 (439/444)	1.1 (5/444)	
Minced beef minced on premises?	Yes	99.3 (142/143)	0.7 (1/143)	No (p=0.6)
	No	98.2 (162/165)	1.8 (3/165)	
Beef burgers prepared on premises?	Yes	97.4 (114/117)	2.6 (3/117)	No (p=0.13)
	No	99.3 (303/305)	0.7 (2/305)	
Fresh or frozen when sampled	Fresh	98.5 (524/532)	1.5 (8/532)	No (p=0.46)
	Frozen	99.5 (208/209)	0.5 (1/209)	
Pre-packaged or loose when sampled	Loose	98.1 (370/377)	1.9 (7/377)	No (p=0.18)
	Pre-packaged	99.5 (364/366)	0.5 (2/366)	
Establishment type	Catering	98.4 (316/321)	1.6 (5/321)	No (p=0.51)
	Retail	99.1 (428/432)	0.9 (4/432)	
Temperature of storage or display	5°C or less	98.9 (639/646)	1.1 (7/646)	No (p=0.28)
	Greater than 5°C	97.6 (83/85)	2.4 (2/85)	

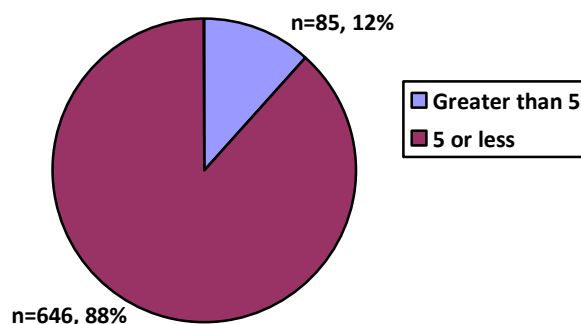
* Fisher's Exact Test (2 sided p-value)

Temperature of storage or display

At the time of sample collection, the temperature of the storage or display unit was measured by the EHO. The recommended storage or display temperature for chilled foods, such as raw meat, is 5°C or less because low temperatures prevent, or slow down, the growth of pathogens and spoilage microorganisms (NSAI 2007a, b).

For this survey, the storage or display temperature was stated for 731 samples (Figure 1). While, 88% (646/731) were stored at the recommended temperature, 85 samples (12%) were stored at temperatures greater than 5°C (ranging from 5.1°C to 13.8°C), which could permit the growth of *Salmonella* or VTEC if present. In fact, 25% (3/12) of samples in which VTEC were detected, were stored at temperatures greater than 5°C; i.e. 5.1, 7.4 and 11°C (Table 3). Even though the percentage of unsatisfactory samples was higher for samples stored or displayed at temperatures greater than 5°C (2.4%) than those at 5°C or less (1.1%), this difference was not statistically significant (Table 4). While temperature abuse does not cause minced beef or beef burgers to become contaminated with *Salmonella* or VTEC, it does increase the risk to health as it may allow the number of pathogens present to increase.

Figure 1: Temperature of storage or display (°C)



Type of packaging

Information on whether the beef was pre-packaged or loose at the time of sampling was provided for 743 samples: 377 (51%) were loose and 366 (49%) were pre-packaged. Information on the type of packaging used was provided for 362 samples. The type of packaging was recorded as vacuum (VAC) packaging for 29% (n=104) of these samples; as modified atmosphere packaging (MAP) for 16% (n=57) of samples or as 'other' for 56% (n=201) of samples. In this survey, three samples of the 'unsatisfactory' samples were described as being pre-packaged at the time of sampling. The type of packaging was recorded as 'other' for two samples and was not stated for the third. Cagney *et al* (2004) found *E. coli* O157 in fresh, frozen, pre-packaged and loose beef and reported that neither freezing nor MAP prevented the survival of *E. coli* O157 in minced beef and burgers.

Date of minimum durability

Pre-packaged minced beef and beef burgers must be labelled with a date of minimum durability in line with the requirements of Council Directive 2000/13/EC on the labelling, presentation and advertising of foodstuffs (European Commission 2000). The results of this survey show that manufacturers are largely complying with this legal requirement, as the date of minimum durability was labelled on 98% (357/366) of pre-packaged samples.

CONCLUSION

Both *Salmonella* and VTEC were detected in samples of raw minced beef and beef burgers collected for this survey. Although all the samples of beef collected for this survey were intended to be cooked before eating, the presence of these pathogens reaffirms the potential for human illness, either through cross-contamination or undercooking.

It is therefore recommended that minced beef and beef burgers are fully cooked (reach a core temperature 75°C) before eating to kill any pathogens that may be present. It is strongly advised that the following types of people *only* eat minced beef or beef burgers that are well cooked:

- Children younger than 5 years of age
- Pregnant women
- People older than 65 years; and
- People undergoing treatments or who have medical conditions which impair their immune system

These people are more vulnerable to infection by VTEC or *Salmonella* and also to the more serious consequences of infection by these bacteria (Lund and O'Brien 2011).

In some European countries, minced meat and beef burgers are consumed raw or undercooked – in dishes such as steak tartare, steak haché, carne cruda, filet américain and tartarmad – but this practice is not common in Ireland. A survey of Irish consumer cooking practices showed that 87% of consumers cook beef burgers 'well done', 12% cook them 'medium' and 1% cook them 'rare' (Teagasc 2005).

A quantitative microbial risk assessment on *E. coli* O157 in beef burgers produced in the Republic of Ireland found that burgers cooked rare (mean internal temperature 5.4°C ± 2°C) constituted a significant risk to the consumer (Duffy *et al* 2006). Investigations of a salmonellosis outbreak in Norway revealed that the four people who fell ill had tasted raw minced beef during its preparation (Isakbaeva *et al* 2005). Case-control studies in France, where the consumption of raw or undercooked minced beef and beef burgers is more common place than in Ireland, identified eating undercooked minced beef and beef burgers as a major risk factor for VTEC-confirmed HUS (French multi-agency outbreak investigation team 2005) and *Salmonella* infection (Haeghebaert *et al* 2001) in children.

This survey showed the beef was stored at temperatures greater than 5°C in a number of retail or catering establishments. This included three samples in which VTEC were detected. Raw meat should be stored at a temperature of 5°C or less in order to prevent or slow down the growth of pathogens that may be present.

The date of minimum durability was labelled on 98% (357/366) of the pre-packaged samples of minced beef and beef burgers, showing that most food business operators complied with this legal requirement.

Finally, 83% (10/12) of VTEC detected in this survey were a serogroup other than *E. coli* O157. The serogroups detected were: O6, O157, O8, O130, O145, O149, O166 and a VTEC O-unidentifiable. Two of these serogroups – *E. coli* O157 and *E. coli* O145 – have been associated with illness in Ireland, but the virulence potential of the other six VTEC serogroups is unknown. If food business operators or official agencies consider it appropriate to test raw minced beef or beef burgers for VTEC, regard should be given to testing for a range of serogroups – in particular those identified by EFSA as being the most frequently linked to human illness (O157, O26, O103, O91, O145 and O111).

RECOMMENDATIONS

As both *Salmonella* and VTEC were detected in raw minced beef and beef burger samples collected for this survey, the following recommendations are made:

1. Raw minced beef and beef burgers should be fully cooked before consumption (to a core temperature of 75°C, or an equivalent time/temperature combination). This is of particular importance for people more vulnerable to infection, e.g. children younger than 5 years of age; pregnant women; people older than 65 years; and people undergoing treatments or who have medical conditions which impair their immune system
2. In retail and catering establishments, raw minced beef and beef burgers should be stored or displayed at 5°C or less
3. If food business operators or enforcement officers consider it appropriate to test raw minced beef or beef burgers for VTEC, consideration should be given to testing for a range of serogroups; in particular O157, O26, O103, O91, O145 and O111, as these serogroups are recognised to cause most cases of human VTEC infection in the EU



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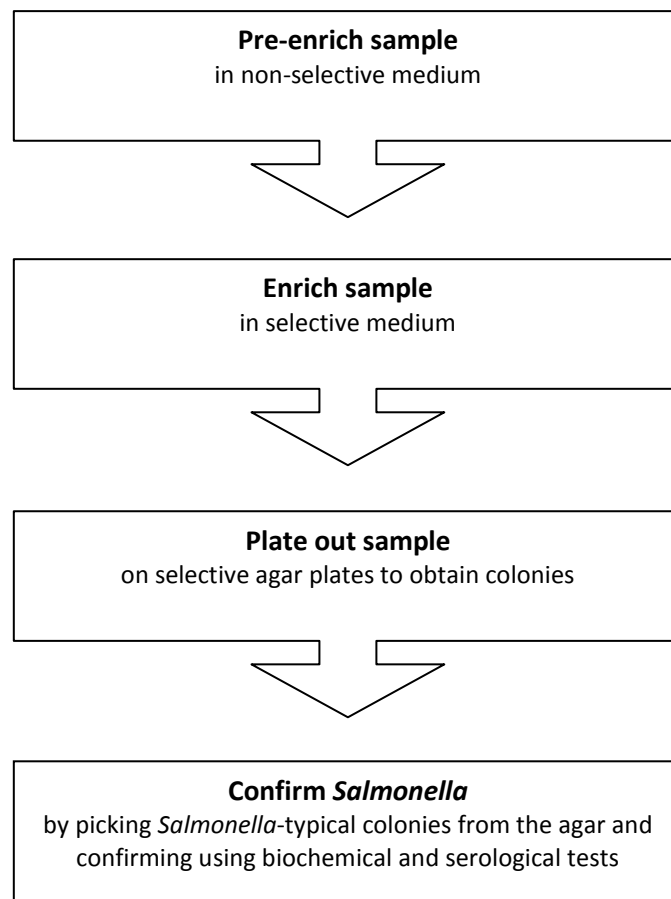
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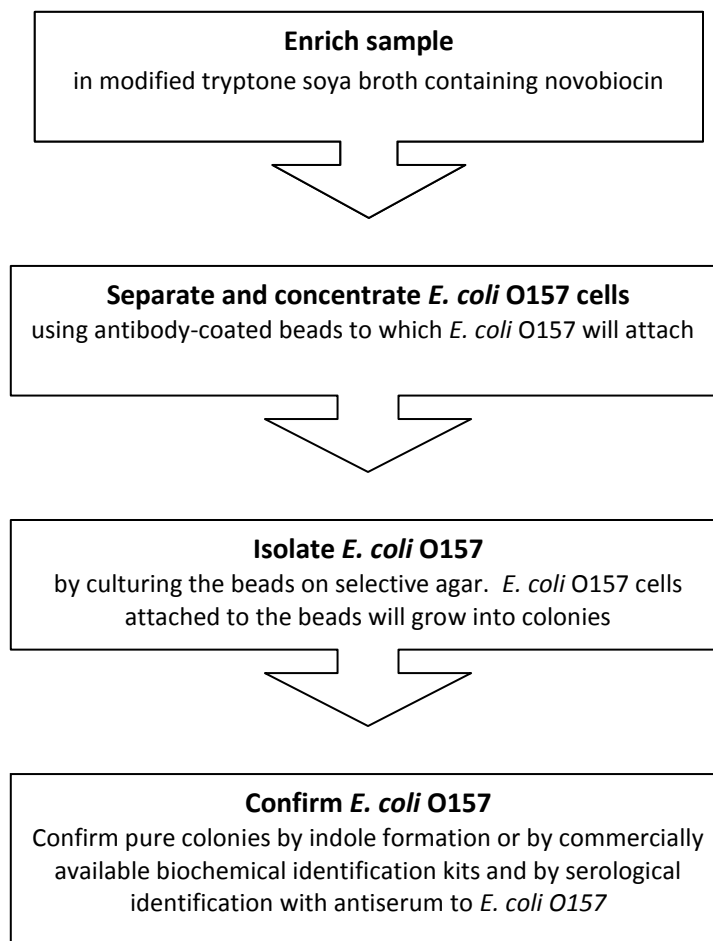
APPENDIX 1: METHOD USED TO DETECT *SALMONELLA*

The standard EN/ISO 6579 method was used to detect *Salmonella*. The Dublin Public Health Laboratory used VIDAS detection with confirmation by the ISO 6579 method if required.



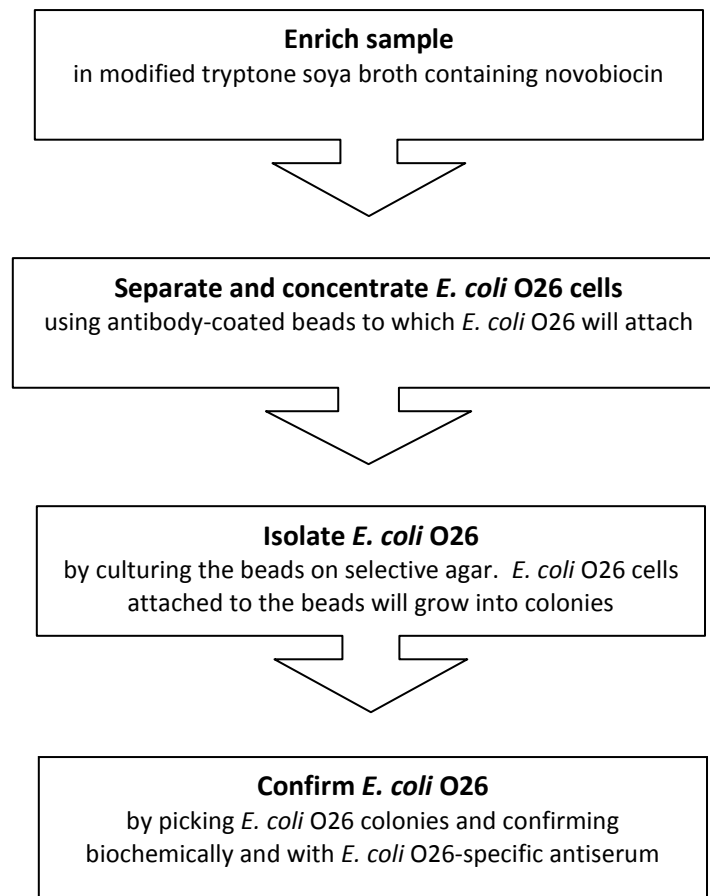
APPENDIX 2: METHOD USED TO DETECT *E. COLI* O157

The standard **ISO 16654:2001** method was used to detect *E. coli* O157. This method is specific to *E. coli* O157 and cannot be used to detect other (non-O157) VTEC. Isolates are confirmed by serology. Dublin Public Health Laboratory also used PCR for detection of VT1 and VT2, with confirmation by serogroup gene if required.



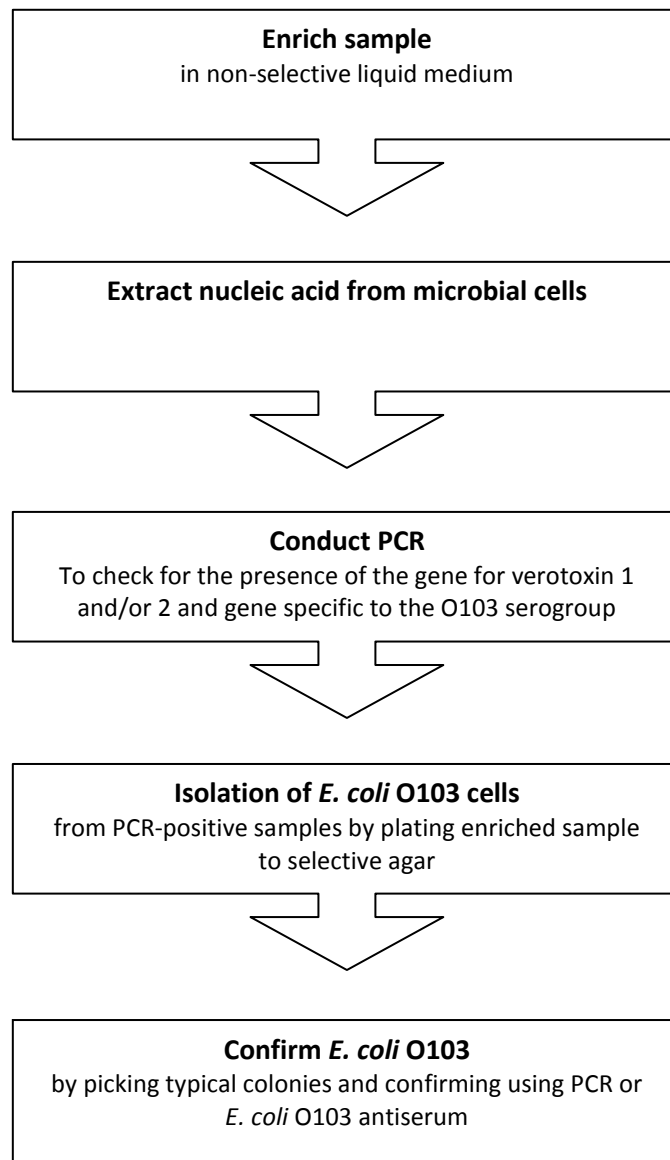
APPENDIX 3: METHOD USED TO DETECT *E. COLI* O26

E. coli O26 was detected using a modified version of the **ISO 16654:2001** method for *E. coli* O157. Isolates are confirmed by serology. Dublin Public Health Laboratory also used PCR for detection of VT1 and VT2, with confirmation by serogroup gene if required.



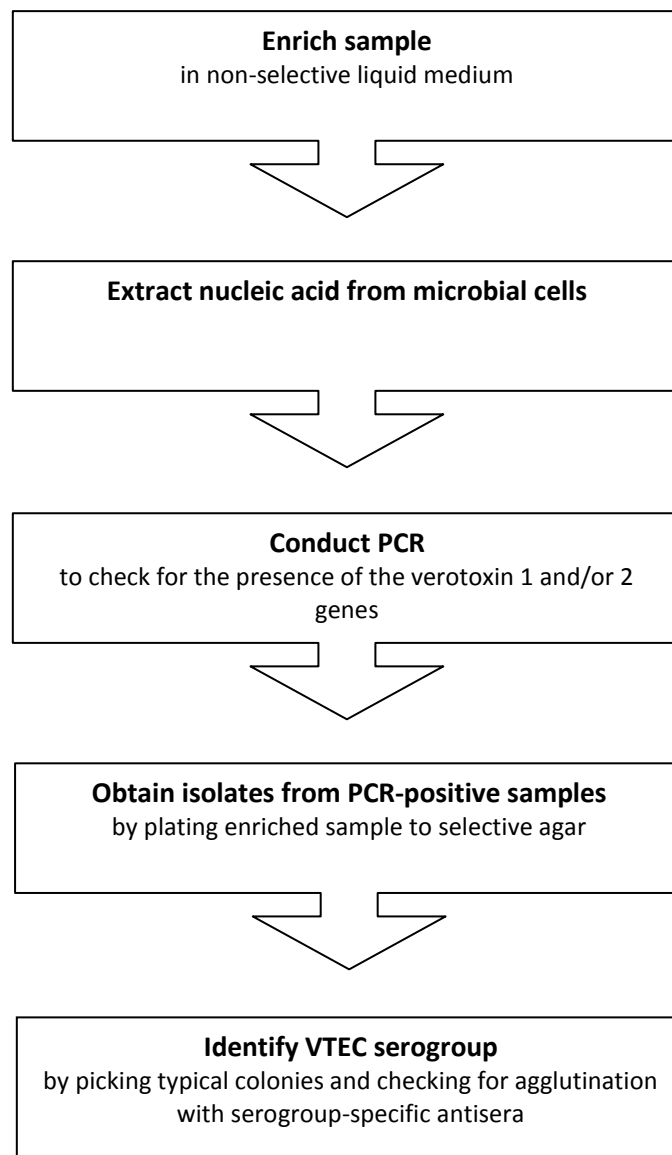
APPENDIX 4: METHOD USED TO DETECT *E. COLI* O103

E. coli O103 was detected using a PCR method specific to *E. coli* O103.



APPENDIX 5: NON-SEROGROUP SPECIFIC PCR METHOD

A non-serogroup specific PCR method to detect VTEC was also used. This method used PCR to detect the genes for *E. coli* verotoxin 1 and/or verotoxin 2 genes in a sample. Samples which were positive for the verotoxin 1 and/or verotoxin 2 genes were then cultured and the resulting isolates confirmed as VTEC using serogroup-specific antibodies.





Abbey Court,
Lower Abbey Street,
Dublin 1.

Advice Line: 1890 336677
Telephone: +353 1 817 1300
Facsimile: +353 1 817 1301
Email: info@fsai.ie
Website: www.fsai.ie