

# Meat technology update

2/12 – October 2012

## Pathogenic Shiga toxigenic *E.coli*—STEC

- Some *E. coli* strains can produce Shiga toxin and are termed Shiga toxigenic *E. coli* or STEC.
- Some of these STEC strains can cause serious gastrointestinal illness, and even death, in humans.
- The US has declared six STEC other than *E. coli* O157:H7 to be adulterants i.e. *E. coli* O26, O45, O103, O111, O121 and O145.
- USDA/FSIS has commenced testing Australian meat imports for these organisms.
- The prevalence of these STEC in Australian beef is low, but likely higher than *E. coli* O157:H7.
- Interventions on the farm, and particularly at the abattoir, are effective in minimising the prevalence of STEC in the final product.
- Isolation of STEC is technically challenging and it may take up to 5 days for a confirmed result to be available.

### Background

*Escherichia coli* are commonly found among the natural microbial flora of the intestinal tract of warm-blooded animals. The majority of *E. coli* strains are not considered pathogenic, but some strains such as *E. coli* O157:H7 can cause serious human illness. *E. coli* O157:H7 is a member of a group of *E. coli* that produce a Shiga toxin referred to as Shiga toxin-producing *E. coli* (STEC). Some STEC can cause gastroenteritis, which in some cases progresses to life threatening complications such as haemolytic uraemic syndrome (HUS). These STEC are known as enterohaemorrhagic *E. coli* or EHEC. The most common EHEC strain is *E. coli* O157:H7. Only a small number of *E. coli* O157:H7 bacteria are required to cause illness, and children and the elderly are particularly susceptible. Cattle have been identified as a major reservoir of *E. coli* O157:H7 and historically disease was associated with the consumption of beef products. More recently disease has been linked to products such as green leafy vegetables that have been contaminated by cattle faeces. The incidence of *E. coli* O157:H7 infections in humans varies between countries with Australia having a notification rate of approximately 0.12 cases per 100,000 per year<sup>(1)</sup> while in the US it is 1.5<sup>(2)</sup> and in Scotland 4.3<sup>(3)</sup> cases per 100,000 per year.

In September 1994, following a large outbreak in the US in 1992–93 due to consumption of undercooked hamburgers, the United States Department of Agriculture, Food Safety and Inspection Service (FSIS) declared *E. coli* O157:H7 to be

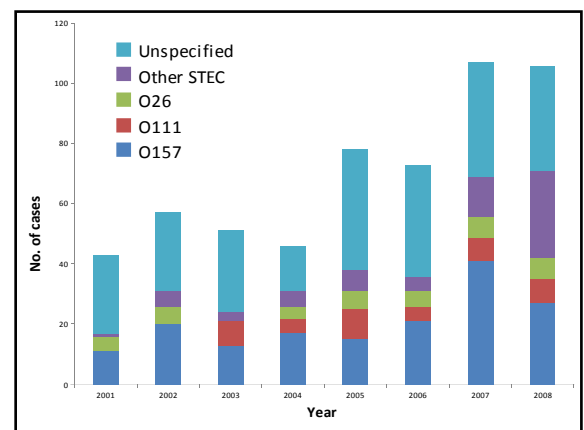


Figure 1: Disease estimates of *E. coli* O157 and non-O157 in the US from 2001 to 2008

an adulterant of raw ground beef and commenced a testing program. The FSIS required raw-beef establishments to reassess their HACCP plans in 2002 to determine if *E. coli* O157:H7 was a hazard reasonably likely to occur and, if so, to implement critical control points (CCPs). The FSIS utilised sampling of raw ground beef and later ground-beef components to verify the establishment's HACCP system was functioning as intended. In response to this, the Australian meat-processing industry, in partnership with the Australian Government Department of Agriculture Fisheries and Forestry (DAFF), implemented a sampling and testing program that led to a reduction in the amount of import testing conducted by FSIS. The Centers for Disease Control (CDC) in the US showed that six STEC serotypes of *E. coli* (O26, O45, O103, O111, O121 and O145) accounted for over 80% of all non-O157:H7 isolates associated with human disease between 2003 and 2006 (Figure 1). As



a consequence of this the FSIS declared these STEC adulterants in ground beef and ground-beef components. As a consequence of this the FSIS has implemented regulatory testing for all 7 STEC strains in raw manufacturing beef trimmings and other ground beef components.

## FSIS and DAFF policies

The Australian meat industry and DAFF and have identified that all 7 STEC serotypes are hazards that are likely to occur in Australia and, therefore, they should be addressed in an establishment's HACCP plan. As all the serotypes have similar growth characteristics, the effectiveness of interventions is expected to be the same for all serotypes. Critical control points (CCPs) identified for *E. coli* O157:H7 are expected to adequately control the other serotypes; however, the ecology of the serotypes in animals may mean that certain types of stock are more at risk of contamination.

Australia produces high-quality product through careful attention to pre-slaughter and processing practices and does not generally rely on interventions such as decontamination to control hazards on meat. While DAFF continues to highlight that there are fundamental differences between the Australian and US meat industries and a lower level of microbial hazards on Australian meat, product may be occasionally contaminated with STEC and this contamination detected during port-of-entry (POE) testing.

FSIS began testing lots of manufacturing trim at POE for the seven STEC (O157:H7, O26, O45, O103, O111, O121 and O145) on 4 June 2012. Testing is limited to product with a slaughter date on or after the 4 June 2012. DAFF has provided some initial feedback to the FSIS on actions Australia is taking to address STEC. These actions include:

- undertaking of a regulatory baseline survey;
- industry testing of US export lots;
- increased verification testing;
- industry reassessment of their HACCP programs for control of STEC.

Once the FSIS position on STEC testing is known, DAFF will seek an equivalence arrangement with FSIS to ensure that actions taken by the FSIS in the advent of a port-of-entry (POE) detection are limited to the tested lot.

The Australian industry has delayed implementation of any regulatory STEC program as the FSIS has placed a 90-day (from 4 June 2012) moratorium on follow-up action taken in the event of a regulatory detection (including POE detections) of any of the STEC under investigation. During this time the FSIS will conduct testing on

domestic and imported product. Any product in which STEC is detected will be declared adulterated. It is not clear how FSIS will consider microbiological independence of adulterated lots. FSIS collect follow-up samples for STEC testing in the event of a positive verification test result. This implies that a POE STEC detection will be treated in the same manner as an *E. coli* O157:H7 POE detection.

During the FSIS 90-day moratorium Australian industry must determine if their HACCP plans control these STEC. DAFF will publish a Meat Notice detailing the requirements of any testing program for STEC on its website once the results of the 90-day trial and FSIS's final position on STEC are known. It is likely that such a program will simply be an expansion of the current *E. coli* O157:H7 program to include testing for the other six serotypes.

Australian establishments are testing lots of manufacturing trim exported to the US for STEC under a Market Access Advice issued by DAFF. This program is not under DAFF supervision and product is not certified by DAFF as having been tested for STEC. DAFF continues to work with industry to determine the most suitable disposition of product tested under this program. While the prevalence of STEC in manufacturing beef appears to be lower in Australia compared to the US, it is higher than the incidence of *E. coli* O157:H7 at around 1%. This means that detection at POE is possible even when STEC has not been detected during sampling and testing in Australia.

## STEC through the slaughter process

Little is known about the ecology of non-O157 STEC. The following information applies to O157. As with other *E. coli*, pathogenic STEC originate in the intestinal tract of sheep and cattle and they are as likely to occur in grass-fed and organically produced stock as in grain-fed animals. They tend to be more associated with faeces than rumen fluid and may be transferred to the hide and fleece and oral cavities of stock by contact with faeces, water troughs, the general environment and grooming. There is then the potential for organisms to be transferred to the carcass surface during the slaughter and dressing process. Occasionally, an individual animal may carry a very high load (10,000 cfu/g or greater) of O157 in its faeces. This animal, known as a 'supershedder', is more likely to contaminate the hide and oral cavities of other members of its group and provide the greatest risk for meat contamination. When this group is processed through the abattoir, there is a higher chance of dressed carcasses being positive for O157. The reasons why some animals on occasion shed high numbers of O157 are unknown, but reducing these numbers will lead to lower contamination of meat and meat products and reduce the risk to human health.

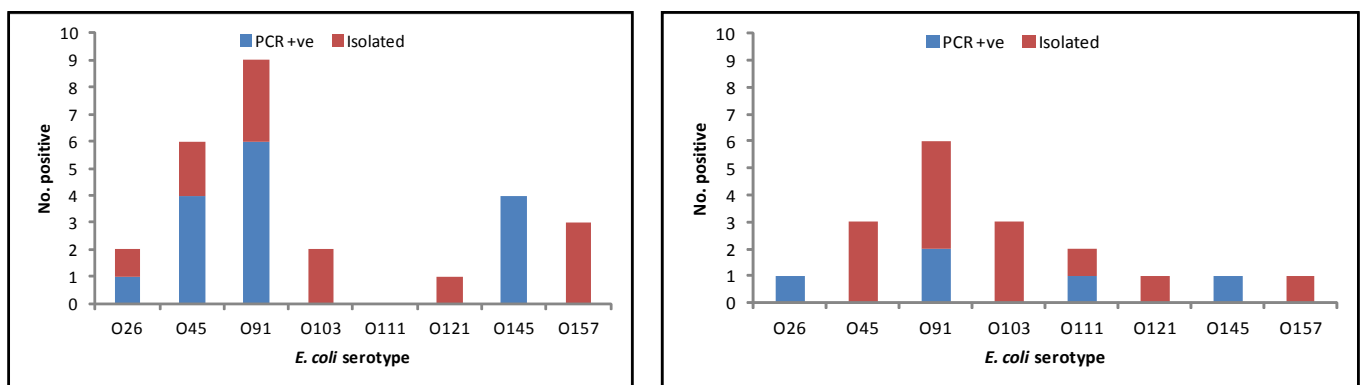


Figure 2: Number of grain-fed (left panel) and grass-fed (right panel) faecal samples testing positive or yielding isolates of the target serotypes during 2008-9

Limited data exists on the prevalence of STEC in cattle and even less in other species. In a study in Australia over an eight-month period in 2008 and 2009, faecal samples were collected from the rectal end of the intestinal tract of 300 grass-fed and grain-fed cattle at slaughter. Of the 300 faecal samples, 30 tested positive for the presence of a Shiga toxin gene, an additional virulence marker, *eae* and for the presence of at least one STEC serotype using real-time PCR. Figure 2 shows the number of grain-fed and grass-fed samples testing positive or yielding isolates of the targeted serotypes. Importantly, however, none of the isolates harboured the necessary combination of virulence markers (i.e. *stx* and *eae*) and were not classified as STEC.

A study in 2011–2012 was conducted to provide an initial estimate of the prevalence of non-O157 STEC strains in Australian manufacturing beef, and to gain an understanding of the performance of commercially available screening tests. From 1,029 samples of beef trimmings (375g) only one positive screening test was confirmed to contain STEC (*E. coli* O26). One sample with a negative screening test result was also confirmed to contain a STEC (*E. coli* O26). The prevalence of non-O157 STEC strains in Australian beef was estimated to be approximately 0.2%. In a US study, 4,133 ground-beef samples were collected from 18 commercial processors, and pathogenic STEC were isolated from 0.2% of samples.

In the Australian baseline survey conducted in 2004, *E. coli* O157:H7 was recovered from one beef carcass from 1155 samples (0.1%) and from 6 out of 1117 sheep carcass samples (0.6%). No *E. coli* O157 were detected in 1082 samples of frozen manufacturing beef and one from 557 samples of frozen sheep meat (0.2%). Results from the 2011 baseline survey indicated a similar prevalence of 0.3% on sheep leg samples and 0.2% on shoulder samples and no *E. coli* O157:H7 on frozen beef primals.

Dairy cattle and calves may be more significant reservoirs of STEC. This is relevant as the meat from culled cows and calf trimmings is mostly used for processing into ground beef. Studies of dairy cattle in the US have reported prevalence of non-O157 STEC in faeces of up to 22%. A study of animals on a dairy farm in Australia over a year showed a low prevalence of *E. coli* O157 in faeces for most of the year, but a sudden increase in one month. If cattle from this farm had been slaughtered during this outbreak, there would have been a higher chance of carcasses being contaminated.

The rate of faecal shedding of STEC by dairy cattle can vary markedly between farms and between cattle of different ages. Calves have a higher incidence than milking cows and the general herd, and weaned calves have a higher incidence than pre-weaned calves (Figure 3). The stress of weaning and diet change, an immature immune system and intensive housing of weaned calves are thought to be the main factors leading to a higher incidence of STEC shedding in this group.

## Interventions

Over the last 10–15 years, a large amount of research has taken place into means of reducing *E. coli* O157 in livestock and in ensuring that, if it is present, it is not transferred to the carcass during dressing, or allowed to survive on the chilled carcass. The majority of the research focussed on *E. coli* O157:H7, but the results are assumed to be equally applicable to control of non-O157 STEC. Below is a list of on-farm and in-plant interventions that have been considered worldwide.

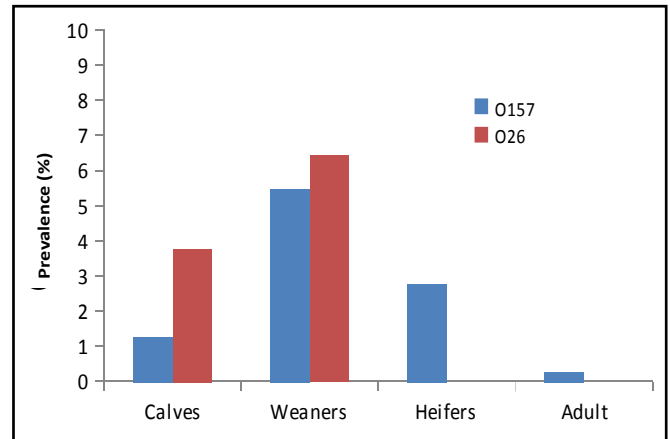


Figure 3: Faecal shedding of *E. coli* O157 and O26 in Australian dairy herds

### On-farm

Methods to control O157 in livestock have met with varying degrees of success, being effective under some conditions, but not others. This is not surprising given the range of production systems and practices in use. Methods trialled have included the following.

- Manipulation of diet to alter the volatile fatty acids and pH in the rumen may reduce the chance of colonisation and shedding.
- Vaccines have been developed for the control of *E. coli* O157:H7. While showing promise, none of the vaccines has been completely successful in eliminating the carriage of this pathogen. Vaccines for a specific STEC serotype are unlikely to be effective in controlling other serotypes.
- Probiotics, which are live microorganisms that can confer a health benefit, have been administered via the feed.
- Bacteriophages (viruses which infect and kill specific bacteria), have also been administered to target *E. coli* O157:H7. Again this technology is likely to be serotype specific.
- Chemicals such as sodium chlorate have been administered to animals.

### In-plant

A wide range of methods have been developed and applied to the control of *E. coli* O157 during slaughter, dressing and further processing. Many of these are general antimicrobial techniques have been refined for use in the meat-plant environment and tested on pathogenic *E. coli*. These interventions are not meant to be replacements for good hygienic practices and will only provide incremental benefits to the final carcass hygiene. Interventions include:

- stock cleaning by de-dagging and water washing to reduce visible soil and microbial numbers on hides;
- steam vacuuming after hide removal, especially along cutting lines;
- pre-evisceration systems such as acid spray washes;
- carcass decontamination on completion of dressing by:
  - hot water
  - steam
  - radiation
  - antimicrobial chemicals and organic acids;

- effective chilling of the carcass to prevent microbial growth (drying of carcass surfaces during chilling is an effective control for bacterial growth and survival).

One of the most effective methods of minimising microbial levels on carcasses is to follow good manufacturing practices (GMP). This can include ensuring animals are clean, facilities are properly cleaned and sanitised, hand and equipment cleaning facilities are adequate and correctly used, and most importantly that staff are trained in hygienic hide removal and evisceration procedures, trimming and observation/removal of zero-tolerance defects (faeces, ingesta, urine and milk) and in operation of equipment.

The interventions listed above have been found to be effective in reducing the prevalence and the numbers of *E. coli* O157 on carcasses and final product. In most cases a series of rigorously tested and approved treatments used in combination have been shown to reduce the risk associated with these pathogens, but not entirely eliminate them. In evaluating an intervention suitable for your plant, you should consider:

- effectiveness of the intervention (how much reduction in microorganisms can be expected);
- effect on product quality (colour, taste, residues, etc.);
- capital cost;
- operating costs;
- environmental considerations such as water and energy use, and chemical and biological waste;
- acceptability to customers.

A package providing summaries and assessments of a large number of interventions is available on the web site [www.meatupdate.csiro.au](http://www.meatupdate.csiro.au).

## Testing for *E. coli* O157 and STEC

*E. coli* O157:H7 and STEC all have two key virulence markers that are utilised during the screening and confirmation process. The screening process utilises PCR (a molecular-based detection system) to test for the presence of the Shiga toxin genes and an additional virulence marker known as *eae*. Information about the serotypes present in the sample is generated by PCR or is implied following immunomagnetic separation (IMS) for the serotypes of interest. IMS uses

magnetic beads coated with antibodies to the specific *E. coli* serotypes to separate target organisms from the enrichment broth. Once the presence of a Shiga toxin gene, *eae*, and a gene for serotype O157 or target STEC has been detected in an enrichment broth, then it is deemed a potential positive and should be sent to a confirmation laboratory for further analysis. Confirmation laboratories will use IMS and PCR to determine if the enrichment broth contains *E. coli* of the appropriate serotype carrying a Shiga toxin gene and *eae*. Confirmation of STEC isolates is technically challenging and may take up to five days for a result to be confirmed. A number of commercially available systems for conducting in-plant screening for *E. coli* O157 and STEC have been approved for use by DAFF. Further information about these systems can be found on the DAFF website (<http://www.daff.gov.au/aqis/export/meat/elmer-3/approved-methods-manual/summary>).

CSIRO acknowledges the assistance of Ian Jenson of MLA and Paul Vanderlinde of DAFF in preparation of this publication.

## Further reading

CSIRO *Meat Technology Update* 6/03, *E. coli*, *E. coli* O157 and Salmonella.

Draft risk profile for pathogenic non-O157 Shiga toxin-producing *Escherichia coli* (non-O157 STEC). USDA Food Safety and Inspection Service, May 2012, available at: [http://www.fsis.usda.gov/PDF/Non\\_O157\\_STEC\\_Risk\\_Profile\\_May2012.pdf](http://www.fsis.usda.gov/PDF/Non_O157_STEC_Risk_Profile_May2012.pdf).

Pathogenic Shiga toxin producing *E. coli* (pSTECs) other than O157 (non-O157 STECs) in manufacturing beef. Baseline survey and method comparison. MLA, Feb 2012.

1 Vally et al. 2012, BMC Public Health, 12:63  
2 Gould et al. 2009 Clin Infect Dis 49:1490  
3 Pearce et al. 2009 BMC Microbiology 9:276

### Final *Meat Technology Update*

This edition of the *Meat Technology Update* is the final in the series distributed by CSIRO. In the future, technical information will be distributed to the Australian meat industry through the resources of the Australian Meat Processor Corporation and Meat & Livestock Australia.

Past editions of the *Meat Technology Update*, *What's New*, *Fact Sheets* and various other scientific reports will continue to be available online.

*The information contained herein is an outline only and should not be relied upon in place of professional advice on any specific matter.*

## Contact us for additional information

Meat Industry Services is supported by the Australian Meat Processor Corporation (AMPC) and Meat & Livestock Australia (MLA).

### Brisbane:

CSIRO Animal, Food & Health Sciences  
PO Box 745  
ARCHERFIELD QLD 4108

### Brisbane:

Neil McPhail  
**T** +61 7 3214 2119  
**F** +61 7 3214 2103  
**M** 0414 336 907  
Neil.McPhail@csiro.au

### Melbourne:

Robyn Warner  
CSIRO Animal, Food & Health Sciences  
Private Bag 16  
WERRIBEE VIC 3030

### Melbourne:

Robyn Warner  
**T** +61 3 9731 3268  
**F**  
**M** 0407 316 760  
Robyn.Warner@csiro.au

This Update and past issues of the *Meat Technology Update* can be accessed at [www.meatupdate.csiro.au](http://www.meatupdate.csiro.au)