

A surveillance study of *E. coli* O111 and *E. coli* O26 in minced beef on retail sale in Ireland

Background

In 1999, the Scientific Committee of the Food Safety Authority of Ireland (FSAI) issued a report entitled '*The Prevention of E. coli O157:H7 Infection: a Shared Responsibility*' (FSAI, 1999). A number of recommendations were made in that report including one for the routine surveillance of VTEC in animals and food.

To address this recommendation, the FSAI commissioned Teagasc to undertake a surveillance study on the incidence of *E. coli* O157:H7 in minced beef on retail sale in Ireland (March 2001 – April 2002). The prevalence of this serotype was reported at 2.8% (Cagney *et al.*, 2004). Although *E. coli* O157 is the main serogroup of the VTEC group which causes infection in humans, other serogroups such as *E. coli* O26 and *E. coli* O111 are emerging as significant. However very little information is available on their prevalence in foodstuffs. This surveillance study was undertaken to assess the prevalence of these serogroups in minced beef on retail sale in Ireland.

This study which was commissioned by the FSAI was undertaken by the Public Health Laboratory SWAHB¹ and The Centre for Food Safety, UCD².

¹ Public Health Laboratory, Cherry Orchard Hospital, Ballyfermot, Dublin 10

² Centre for Food Safety, Faculty of Veterinary Medicine, University College Dublin, Belfield, Dublin 4

Introduction

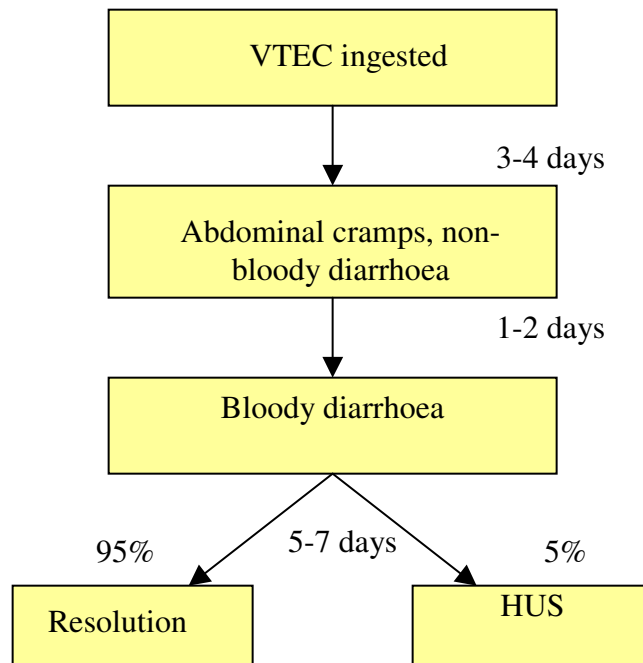
Escherichia coli is a predominant facultative anaerobe of the human colonic flora. The organism typically colonises the infant gastrointestinal tract within hours of life, and thereafter *E. coli* and the host derive mutual benefit (Nataro and Kaper, 1998). Most *E. coli* do not cause disease in humans, but certain types may cause diarrhoeal disease or more serious forms of illness. Among those *E. coli* that may cause serious disease and death are a group called Verocytotoxigenic *E. coli* (VTEC).

VTEC strains are commonly found in the intestines of livestock. Human infections are usually a consequence of consumption of contaminated meat or dairy products which have been improperly cooked or processed, or uncooked vegetable products that have come into contact with manure (Karmali, 1989).

The pathogenicity of VTEC is associated with a number of virulence factors such as verotoxins (VT1 & VT2), *eae* that encodes intimin which is responsible for the attaching and effacing of the organism to the gut epithelial cells and *hly* that encodes enterohaemolysin. Clinical infection is characterised by abdominal cramps followed by bloody diarrhoea. Infection can become life threatening due to the subsequent development of haemolytic-uremic syndrome (HUS) (James *et al.*, 2001) (Fig 1).

VTEC strains can be divided into many sub-groups, referred to as serogroups, based on variations in the structure of the surface of the bacterium. The principal serogroups are defined by chemicals on the surface of bacterial cells known as O-antigens and then subdivided into serotypes on the basis of other structural variations, e.g. H-antigens. The main serotype that frequently produces verocytotoxin and causes the classical illness associated with these pathogens is *E. coli* serotype O157:H7. This serotype was first identified as a human pathogen in 1982 (Mead and Griffin, 1998).

Fig 1: Natural history of infection with VTEC infection (Mead and Griffin 1998)



E. coli O157 is the most commonly isolated VTEC serogroup in the UK, US and Japan, however more than 30 disease causing non-O157 serogroups have been described (Acheson *et al.*, 1998). Non-O157 serogroups which have emerged as a significant cause of human disease include *E. coli* O26 and *E. coli* O111 (Tarr and Neill, 1996). There has been some evidence of these infections in Ireland. An outbreak of *E. coli* O26 occurred in a crèche in 1998 (McMaster *et al.*, 2001). Some non-O157 outbreaks have been linked to cattle (Blanco *et al.*, 1996), however the source of most non-O157 outbreaks is largely unknown. For this reason it was deemed important to evaluate the presence of non-O157 VTEC serotypes in raw minced beef from retail premises in Ireland.

Many publications have detailed detection of *E. coli* O157 by culture methods, however significantly less have detailed detection of *E. coli* O26 and *E. coli* O111. The findings of Hara-Kudo *et al.*, 2000 and Safarikova and Safarik, 2001 among others were taken into consideration when designing the methodology for this survey.

Brief Methodology

A total of 800 samples of raw minced meat were collected from retail premises in the 26 counties in the Republic of Ireland. Retail premises comprised of both supermarkets (10 supermarket chains and a number of independent supermarkets) and butchers (ratio of supermarkets:butchers = 2:1). Both pre-packed (n=352) and loose (n=448) samples were collected (ratio of pre-packed:loose = 1:1.27).

Sampling was carried out in each quarter (i.e. 200 samples were collected in each 3 month period). The sampling schedule was designed so that samples were obtained from each retail premises in each quarter (i.e. each premises was sampled 4 times in the course of the study).

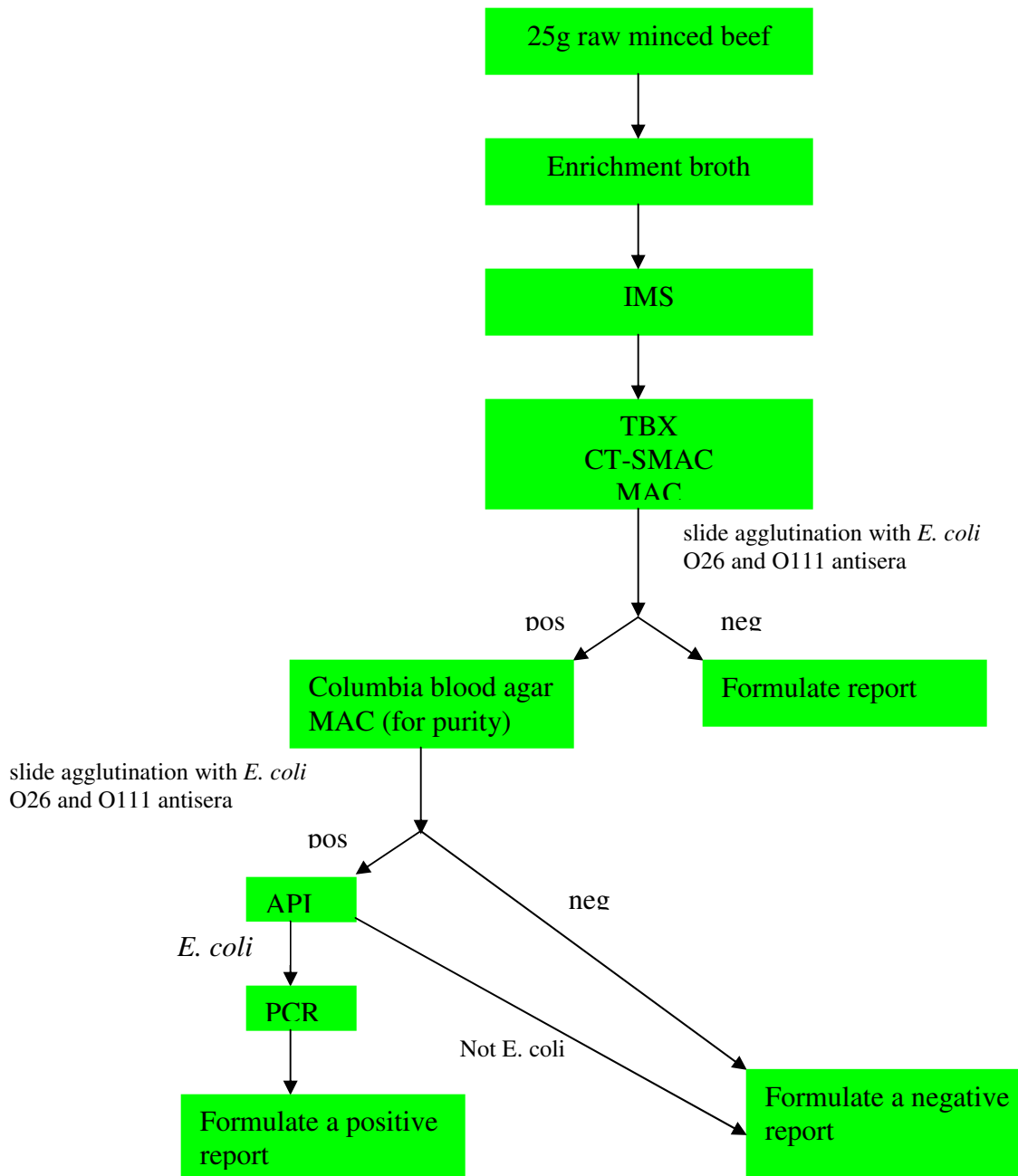
Sampling periods:

Quarter 1:	31/3/03 – 9/6/03
Quarter 2:	16/6/03 – 22/9/03
Quarter 3:	29/9/03 – 1/12/03
Quarter 4:	8/12/03 – 22/2/04

Samples were transported to the PHL-SWAHB in cool boxes fitted with calibrated temperature probes. The temperature of each sample was taken on arrival at the PHL and recorded. The sell by/use by date on each sample was also recorded.

A flow chart of the methodology used to analyse the samples is represented in Figure 2. These methods were sensitive and specific and had been previously validated.

Figure 2: Methodology used to analyse the raw minced meat samples



Results

E. coli O111:

- *E. coli* O111 was not detected in any sample.

E. coli O26:

- *E. coli* O26 was detected in 2 samples, i.e. incidence of 0.25% (2/800).

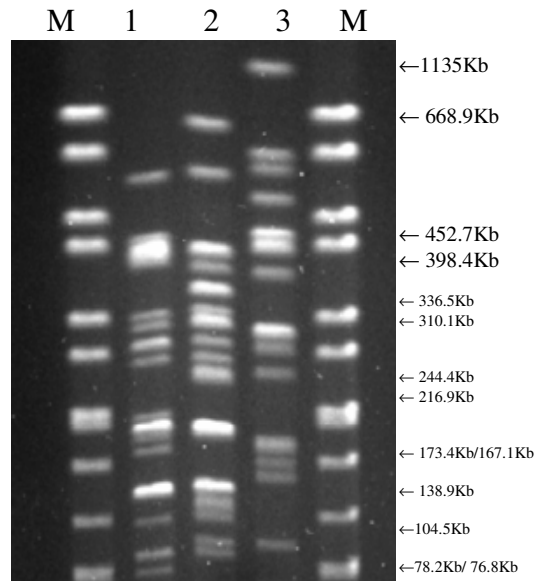
Information relevant to these samples is outlined in table 1:

Table 1: Information relevant to the 2 samples in which *E. coli* O26 was detected

Sample number	Sample nature	Sample location	Sampling quarter
1	Pre-packed	x	3
2	Pre-packed	y	4

- PCR revealed that both isolates were negative for verotoxin (VT1 and VT2) genes. Therefore, there was no public health risk associated with these isolates.
- Both isolates were subject to Pulsed-Field Gel Electrophoresis (Fig 3) to determine if they were related at a genetic level. Both isolates had different PFGE patterns. This showed that the isolates were unrelated and did not have a common source. The patterns were interpreted according to guidelines by Tenover *et al* 1995.

Figure 3: PFGE of two culture positive *E. coli* O26 isolates



Lane M: *Salmonella* Branderup,
Lane 1: isolate 1,
Lane 2 : isolate 2,
Lane 3: Pos Control NCTC *E. coli* O26

Discussion

This national survey of raw minced beef at retail level was reassuring, i.e. *E. coli* O111 was not detected in any sample and *E. coli* O26 was detected in only 2 samples (incidence rate of 0.25%). Of particular reassurance was the finding that the *E. coli* O26 isolates were negative for Verocytotoxin genes and therefore posed no potential public health risk. Despite these findings it is imperative that controls continue to be implemented at relevant stages through the food chain, i.e. from on-farm to consumers.

The incidence of these non-O157 serotypes in raw minced meat is lower than that reported for O157 (2.8%) in a previous survey (Cagney *et al.*, 2004). This finding correlates with clinical findings at PHL-SWAHB (2002 unpublished data) that the incidence of *E. coli* O157 was 10 times greater than the incidence of non-O157 VTEC.

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