



Title:

Surveillance of Dairy Production Holdings Supplying Raw Milk to Farmhouse Cheese makers for Verocytotoxin producing *E. coli* O157 and other VTEC.

Project Partners:

Food Safety Authority of Ireland
Irish Farmhouse Cheesemakers Association
Veterinary Section, Cork County Council

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Introduction

In March 2004, the FSAI commissioned a study, entitled; 'Surveillance of Dairy Herds Supplying Raw Milk to Farmhouse Cheese makers for verocytotoxin producing *E. coli* O157 and other verocytotoxin producing Ecoli (VTEC) serotypes. Examination of the samples was undertaken at the Veterinary Food Safety Lab, Cork County Council and Centre for Food Safety, located in the School of Veterinary Medicine at University College Dublin.

Following discussions with Cáis members (Irish Farmhouse Cheese Makers Association) and individual cheese producers, it was agreed that participation of herd owners would be on a voluntary basis. All herd owners showed great enthusiasm towards their involvement in this surveillance project. This monitoring programme, based on milk filter residue analysis, undertaken during a number of seasonal cycles has established baseline data on the prevalence and characteristics of VTEC organisms in lactating animals (bovines, ovines, caprines) supplying the farmhouse cheese sector.

Background

Escherichia coli (*E. coli*) O157 is but one of a group of verocytotoxin-producing *E. coli* (VTEC) associated with severe disease in humans. Mild infection may result in bloody or non-bloody diarrhoea but in acute cases serious complications like haemolytic uremic syndrome (HUS) may develop (Mead and Griffin., 1998). Although, *E. coli* O157 is the most renowned VTEC, other serogroups, including O26, O111, O103, O145 and O121 have the potential to cause serious human illness (Bonnet et al., 1998; Caprioli et al., 1997). A number of non-O157 VTEC infections in humans (mostly in children) have been reported in Ireland. Human cases attributed to O26 have already been documented in the North West region of Ireland (McMaster et al., 2001) A recent sporadic case involving a farming family in the Munster region (on-farm investigation undertaken by the Veterinary Section, CCC) highlighted the fact that ovine faeces and equine faeces can be a potent source of highly virulent *E. coli* organisms. However, the source of most non-O157 VTEC outbreaks is largely unknown.

Healthy domestic animals, in particular, ruminants like cattle, sheep and goats can harbour O157 VTEC and other VTEC in their faeces. These species are regarded as natural reservoirs of these organisms. Milk filters residue can be used efficiently and cost effectively to monitor faecal shedding, direct milk secretion and environmental contamination for these organisms.

Methodologies

This surveillance project involved seventy-four milk production holdings, throughout Ireland. Fifty-six bovine, 13 caprine and 5 ovine herds participated in the project. Cáis members, individual cheese producers and the local authority veterinary officers facilitated in herd participation and with sample collection. The sampling programme was divided into three cycles thereby each farm was tested on three separate occasions (where possible).

All milk filter residue samples were analysed by conventional microbiological protocols. Immunomagnetic separation (IMS) was used to test for the presence of the *E. coli* strains, on a serogroup-specific basis. Any suspect colonies were confirmed using molecular methods such as polymerase chain reaction (PCR). Further characterisation to identify markers of virulence and pathogenicity was undertaken on all serogroups isolated. Each isolate was examined for the presence of both verocytotoxins (*vt1/vt2*), the hemolysin encoding gene (*hlyA*) and the gene encoding attaching and effacement (*eae*).

Results

In this study, five verocytotoxigenic *E. coli* O157 isolates were detected. Another twenty-one *E. coli* O157 isolates were found but these were deficient for the verocytotoxin genes. Seventeen *E. coli* O26 isolates have been detected, four of which were verocytotoxigenic, seven isolates contained the attaching and effacement gene (*eae*) only, and six isolates expressed none of the virulence factors examined. No *E. coli* O111 isolates were detected in this study, (Table 1 and 2). While, product and bulk milk examinations have been carried out on all VTEC positive production holdings, no VTEC organisms were detected.

All herd-owners were notified of the laboratory results. A back up advisory service has been provided by Cáis specialist adviser, Sara McSweeney located at Teagasc Moorepark and the Veterinary Department of Cork County Council.

On-going work

All isolates recovered are being phenotypically characterised to determine antimicrobial resistance profiles and genotypically characterised, using pulsed-field gel electrophoresis (PFGE) or multiple-locus variable-number of tandem repeat analysis (MLVA). A Bionumerics Library is been established to enable future comparative analysis of human, animal and environmental isolates.

Conclusions

An unexpected finding in this study has been the number of *E. coli* O157 isolates that were VTEC deficient and the number of O26 isolates that were VTEC negative, but showed other virulence factors (Table 1 and 2). A database of these isolates has been established and these strains will be further characterised, to establish their potential pathogenicity and their flagellar gene status.

This surveillance project has identified a number of VTEC positive herds, which may be supplying milk for unpasteurised cheese and also for the production of unpasteurised ice cream. Although, all products tested from these holdings are VTEC negative to date, routine surveillance of milk production holdings for VTEC and other such pathogens continues to be of public health importance.

This research surveillance project has resulted in technology transfer and development of molecular methodologies, which enable a rapid, reliable multi-disciplinary response for the investigation of sporadic VTEC outbreaks.

Table 1: Summary table of the virulence characteristics in the *E. coli* isolates.

Virulence markers/genes	<i>E. coli</i> O111	<i>E. coli</i> O26	<i>E. coli</i> O157
<i>vt1</i> , <i>eae</i> & <i>hlyA</i>	0	4	0
<i>vt2</i> , <i>eae</i> & <i>hlyA</i>	0	0	5
<i>eae</i> only	0	7	0
No virulence factors	0	6	21
Total	0	17	26

Table 2. Characteristics of the *E. coli* O157 isolates

Isolate Number	Source	Serogroup	Virulence Markers			
			<i>vt1</i>	<i>vt2</i>	<i>eae</i>	<i>hlyA</i>
VTEC-6a	Bovine Milk Filter	<i>E. coli</i> O157	-	-	-	-
VTEC-16a	Bovine Milk Filter	<i>E. coli</i> O157	-	-	-	-
VTEC-26a	Bovine Milk Filter	<i>E. coli</i> O157	-	-	-	-
VTEC-38a	Bovine Milk Filter	<i>E. coli</i> O157	-	-	-	-
VTEC-46a	Bovine Milk Filter	<i>E. coli</i> O157	-	+	+	+
VTEC-47a	Bovine Milk Filter	<i>E. coli</i> O157	-	-	-	-
VTEC-48a	Bovine Milk Filter	<i>E. coli</i> O157	-	+	+	+
VTEC-54a	Bovine Milk Filter	<i>E. coli</i> O157	-	-	-	-
VTEC-56a	Caprine Milk	<i>E. coli</i> O157	-	-	-	-
VTEC-60a	Ovine Milk Filter	<i>E. coli</i> O157	-	-	-	-
VTEC-65a	Caprine Milk Filter	<i>E. coli</i> O157	-	+	+	+
VTEC-68a	Bovine Milk Filter	<i>E. coli</i> O157	-	-	-	-
VTEC-13b	Caprine Milk Filter	<i>E. coli</i> O157	-	+	+	+
VTEC-13b	Caprine Milk Filter	<i>E. coli</i> O157	-	-	-	-
VTEC-25b	Bovine Milk Filter	<i>E. coli</i> O157	-	+	+	+
VTEC-25b	Bovine Milk Filter	<i>E. coli</i> O157	-	-	-	-
VTEC-9b	Bovine Milk Filter	<i>E. coli</i> O157	-	-	-	-
VTEC-10b	Bovine Milk Filter	<i>E. coli</i> O157	-	-	-	-
VTEC-38b	Bovine Milk Filter	<i>E. coli</i> O157	-	-	-	-
VTEC-52b	Bovine Milk Filter	<i>E. coli</i> O157	-	-	-	-

VTEC-26b	Bovine Milk Filter	<i>E. coli</i> O157	-	-	-	-
VTEC-3c	Bovine Milk Filter	<i>E. coli</i> O157	-	-	-	-
VTEC-69b	Bovine Milk Filter	<i>E. coli</i> O157	-	-	-	-
VTEC-26c	Bovine Milk Filter	<i>E. coli</i> O157	-	-	-	-
VTEC-25c	Bovine Milk Filter	<i>E. coli</i> O157	-	-	-	-
VTEC-3c-2	Bovine Milk Filter	<i>E. coli</i> O157	-	-	-	-
VTEC-6a	Bovine Milk Filter	<i>E. coli</i> O26	-	-	+	-
VTEC-26a	Bovine Milk Filter	<i>E. coli</i> O26	-	-	+	-
VTEC-51a	Bovine Milk Filter	<i>E. coli</i> O26	-	-	-	-
VTEC-64a	Bovine Milk Filter	<i>E. coli</i> O26	-	-	-	-
VTEC-65a	Caprine Milk Filter	<i>E. coli</i> O26	+	-	+	+
VTEC-36b	Caprine Milk Filter	<i>E. coli</i> O26	+	-	+	+
VTEC-11b	Bovine Milk Filter	<i>E. coli</i> O26	-	-	+	-
VTEC-54b	Bovine Milk Filter	<i>E. coli</i> O26	-	-	+	-
VTEC-23b	Bovine Milk Filter	<i>E. coli</i> O26	-	-	+	-
VTEC-68b	Bovine Milk Filter	<i>E. coli</i> O26	-	-	+	-
VTEC-15c	Bovine Milk Filter	<i>E. coli</i> O26	+	-	+	+
VTEC-16c	Bovine Milk Filter	<i>E. coli</i> O26	-	-	+	-
VTEC-68c	Bovine Milk Filter	<i>E. coli</i> O26	-	-	-	-
VTEC-70c	Bovine Milk Filter	<i>E. coli</i> O26	+	-	+	+
VTEC-70c	Bovine Milk Filter	<i>E. coli</i> O26	-	-	-	-
VTEC-45c	Bovine Milk Filter	<i>E. coli</i> O26	-	-	-	-
VTEC-69c	Bovine Milk Filter	<i>E. coli</i> O26	-	-	-	-

References

Bonnet, R., S. Souweine, G. Gauthier, C. Rich, V. Livrelli, J. Sirot, B. Joly and C. Forestier. 1998. Non-O157: H7 Stx2-producing *Escherichia coli* strains associated with sporadic cases of hemolyticuremic syndrome in adults. J. Clin. Microbiol. 36:1777-1780.

Caprioli, A., A. E. Tozzi, G. Rizzoni, and H. Karch. 1997. Non-0157 Shiga toxin-producing *Escherichia coli* infections in Europe. Emerg. Infect. Dis. 3:578-579.

McMaster, C., Roch, E.A., Willshaw, G.A., Doherty, A., Kinnear, W., Cheasty, T., 2001. Verocytotoxin-producing *Escherichia coli* serotype O26:H11 Outbreak in an Irish crèche. Eur. J. Clin. Microbiol. Infect. Dis. 20, 430-432.

Mead, P.S. and P.M. Griffin. 1998. *Escherichia coli* O157:H7. Lancet. 352:1207-1212.

