3rd SCIEH
Verocytotoxigenic \textit{E.coli} Update
23 November 1999

Introduction

In his preface to the proceedings of the second SCIEH Verocytotoxigenic \textit{E.coli} Workshop held in January 1997, my predecessor, Professor Dan Reid, referred to Scotland’s unenviable record with \textit{E.coli} O157. He recalled the large milk-borne outbreak in 1994 and the Central Scotland outbreak of November 1996.

Much has happened in the intervening two years since the last workshop. What has not happened has been the disappearance of this major threat to public health. It seemed opportune, therefore, to bring the various disciplines together to review progress in this field and discuss where we might be going.

This workshop was sponsored by the Scottish Centre for Infection and Environmental Health (SCIEH) and the Scottish Veterinary and Medical Consortium (SVMC) on Food Safety with considerable support from the Scottish Executive Rural Affairs Department. I am very grateful to Professor Bill Reilly, Deputy Director of SCIEH, not only for arranging the workshop, but also for producing the proceedings so timeously.

It gives me great pleasure to have co-hosted this Update with the Scottish Veterinary Medical Consortium on Food Safety. The ambitious programme reflected the wide range of relevant areas under investigation and the necessity for a multi-disciplinary approach.

Dr Ian G Jones
Director

\begin{quote}
\textbf{The Scottish Veterinary and Medical Consortium (SVMC) on Food Safety}

Tragic events such as the Central Scotland \textit{E.coli} outbreak helped raise awareness of the relationship between food, animals and human health, highlighting the need for stronger links between veterinarians and others in public health. The Scottish Veterinary and Medical Consortium (SVMC) on Food Safety was established to provide a platform for the different medical and veterinary disciplines involved in food safety to discuss and respond to issues such as those raised in the James Report on Food Safety and the White Paper, \textit{The Food Standards Agency - A Force for Change}. The Consortium includes colleagues from public health, veterinary medicine, microbiology, animal diseases research and agricultural backgrounds, providing an ideal framework within which to collaborate on education in food related issues and investigate the pattern of disease and food chains. In addition to the Scottish Centre for Infection and Environmental Health (SCIEH), the Consortium involves the Scottish Agricultural College (SAC), the Moredun Research Institute and Glasgow, Edinburgh and Strathclyde Universities.
\end{quote}
Acknowledgements

Without the considerable efforts of members of staff at SCIEH, the Update itself and the production of the proceedings would not have been possible. In particular we would like to thank Mrs S Jardine, Mrs E Carragher, Ms W Ross, Ms R Shoafenge, Mr M Getty and Mr F Boero.

The generous financial support of the Scottish Executive Rural Affairs Department (SERAD) enabled registration costs to be kept to a minimum and facilitated the production of the proceedings.
Contents

Session 1
Chair - Dr Ian Jones

Central Scotland *E.coli* O157 outbreak: Clinical update Dr WTA Todd 5

*Escherichia coli* O157:H7 - an economic assessment of an outbreak Dr JA Roberts 7

A case-control study of sporadic cases of *Escherichia coli* O157 infection in Scotland Ms M Locking 9

The PHLS Case-control Study of *Escherichia coli* O157 Infection in England Dr GK Adak 11

Session 2
Chair – Dr John Cowden

Vero cytotoxin-producing *Eschericia coli* (VTEC) risk factors in the farming environment Dr R Chalmers 12

HUS surveillance: What does it tell us about VTEC? Dr SO’Brien 14

International surveillance of *E.coli* O157 through Enter-net Mr I Fisher 15

Genetic heterogeneity of *E.coli* O157 in Scotland and its utility in strain typing Dr FM Thomson-Carter 17

Vero cytotoxin-producing *Escherichia coli* O157 in England and Wales: update on laboratory studies Dr HR Smith 19

Session 3
Chair – Professor Andrea Nolan

Improved methods of *E.coli* O157 detection in food Dr ID Ogden 20

Survey of the prevalence of VTEC O157 in raw meats, raw cow’s milk and raw milk cheeses in South-east Scotland Dr J Coia 22

*Eschericia coli* O157: H7 and dairy products in the North-west of England Ms HC Aird 23

Survival of *Escherichia coli* 0157 in water and organic wastes on land: the potential to contaminate untreated private water supplies Dr CW Keevil 24

Session 4
Chair – Professor Bill Reilly

Dispersal of *E.coli* O157 in soil and contamination of water Dr JM Hall (now Ritchie) 26

Survival of *E.coli* O157 in soil and water D R Fenlon 28

*Escherichia coli* in farm animals C S Stewart 30

SAC cattle studies on *Eschericia coli* O157 Mr BA Synge 32

Wellcome Trust Project Prof MEJ Woolhouse 33
**Poster Presentations**

A comparison of Verocytotoxin-producing *Escherichia coli* O157 phage types isolated in England and Wales with those from 13 other European countries: January 1997 to June 1999  
Mr T Cheasty  
Page 34

Mucosal and systemic immune responses to the lipopolysaccharide of *E. coli* O157  
Ms C Currie  
Page 35

The relationship between cattle cleanliness and carcase contamination  
Dr L Humpheson  
Page 36

Novel mucosal vaccination  
Dr JF Huntley  
Page 38

Current Veterinary Laboratory Agency (VLA) surveillance and research on *E. coli* O157  
Mr G Paiba  
Page 40

Toxin sequences in disease causing *E. coli* strains  
Prof AN Turner  
Page 42

**Author contacts**

**Participants**
Central Scotland *E.coli* O157 outbreak: Clinical Update

WTA Todd, S Dundas, Al Stewart, PS Murdoch, SL Hutchinson and AKR Chaudhuri

**Introduction**

The Central Scotland outbreak of *E.coli* O157 infection was centred around Lanarkshire and the majority of severe cases were managed in one institution (Monklands District General Hospital). A consistent clinical approach was observed for these cases, and reliable records and laboratory results were available for a significant proportion of the cases. Although 503 cases were recorded as part of the outbreak, in only 345 was there reliable evidence of the infecting organism being the recognised outbreak strain of *E.coli* O157. Of these, 120 (35%) were hospitalised, 44 (13%) showed evidence of Thrombotic micro-angiopathy (TMA) and 34 (10%) developed full Haemolytic Uraemic Syndrome (HUS) or Thrombotic thrombocytopenic purpura (TTP). Fourteen required dialysis and 20 died.

From a clinical viewpoint, although *E.coli* O157 accounts for only 300 notifications per year in Scotland, the significant percentage developing the potentially life threatening complication of HUS/TTP make this illness very important and of greater clinical importance than other more common food poisoning conditions. The Infectious Disease physician in particular has the opportunity to diagnose the illness and potentially intervene in its progress before complications develop.

For this reason we have retrospectively analysed confirmed cases from the Central Scotland outbreak to assess risk factors for the development of complications. A full analysis of these data will be reported fully elsewhere; however, the trends can be reported here.

**Methods**

A multivariate regression analysis was performed using independent clinical variables and outcome measures. Demographic data, clinical signs and symptoms, pre-morbid illness and laboratory values were all examined by this method for any association with the development of complications (HUS/TTP).

**Results**

Age emerged as an important risk factor, with those at the extremes of age (<5 years or >65 years) having an increased risk of HUS/TTP (odds ratio (OR) 4.35; confidence interval (CI) 1.3-14.4 for both groups).

Of the clinical signs and symptoms, a short incubation period <four days (OR 3.47; CI 1.0-11.7), the presence of a fever on admission (>38°C) (OR 2.57; CI 1.0-6.7) and a significant tachycardia on admission (OR 9.33; CI 1.8-48.9) were the only features that had a significant statistical association with the development of HUS/TTP. Pre-morbid clinical illness had remarkably little association with the development of complications. The only feature that had an association was obvious or inferred hypochlorhydria (OR 6.00; CI 1.9-19.3).

A number of haematological and biochemical parameters as measured within 48 hours of admission were examined and only two gave a statistically significant association with HUS/TTP: the presence of a neutrophil leukocytosis (>15 x 10⁹/l) (OR 8.5; CI 1.5-50) or of hypoalbuminaemia (<35 g/l) (OR 7.2; CI 1.2-42.5) were shown to be independent predictors of the development of complications. Blood urea, creatinine level, lactate dehydrogenase, haemoglobin and platelet count were all indicators of the development of complications but not predictors of that problem.

We also examined the effect of antibiotic use on outcomes during the outbreak. The administration of antibiotics within the four weeks prior to the development of symptoms was associated with the development of complications (OR 5.07; CI 1.5-16.8). Fifteen cases had ciprofloxacin administered according to the BSSI (British Society for the Study of Infection) guidelines¹. Although this group was twice as likely to develop complications as were those who did not receive antibiotics, this association did not reach statistical significance. The clinician faced with cases of *E.coli* O157 infection can, therefore, select those most likely to develop further problems and concentrate efforts on the follow up of such individuals.

The management of the complications once they arise remains controversial. Paediatric practice has been to offer early dialysis, recognising that once complications have arisen 9% of children will develop End-stage Renal Disease and 30% will have persistent abnormalities of renal function. The long-term outlook for adults who have suffered complications is not known. Early in the outbreak it was decided to attempt Therapeutic Plasma Exchange (TPE) in...
any adult who showed signs of TMA. Untreated in the elderly, HUS has a reported mortality of 88% and TPE used in other forms of TMA/HUS has a mortality rate of 31%\(^2\). Thirty-four patients developed TMA; 28 proceeded to formal HUS/TTP. Of these, six were children and therefore not offered TPE. Five patients either died before therapy could be instituted or were too sick to undergo this therapy. There were five deaths in the 16 treated with TPE (mortality rate 31%)\(^3\). There may, therefore, be a relevant therapeutic regime when patients with known *E.coli* O157 infection are recognised to have risk factors or to be developing complications. This method of treatment requires further study, as does the use of antibiotics in *E.coli* O157 infection.

Currently, the Scottish Executive is funding a long-term follow up of cases to examine the likelihood of late complications of this condition.

**References**

Escherichia coli O157:H7 - an economic assessment of an outbreak

JA Roberts and PA Upton

Background

The outbreak of Escherichia coli O157:H7 in May 1994 described in this report was the largest reported milk-borne outbreak on record in the UK and the first reported worldwide to have involved a heat-treated milk supply (1). The outbreak occurred in a rural community to the west of Edinburgh, with a population of approximately 107,000. The size of the outbreak, the severity of the illness and the high costs merited further exploration and this was supported by a grant from the Department of Health.

Aims

The aim was to assess the economic and social impact of the outbreak. The specific objectives included assessing the impact upon cases and their families, on hospitals, primary and community care facilities, laboratories, and public health and environmental health departments during the acute phase of the illness and the subsequent 12 months. Projections of the implications of the illness on the cases over the next 30 years were also made.

Methods

Three surveys of confirmed cases were undertaken using GP notes, hospital records and interviews with cases. Key persons involved in the investigation and control of the outbreak were also interviewed. The impact of the illness on cases and their families was estimated and the resources used to treat cases and to control the outbreak were costed and long-term costs projected.

Case definition

There were 71 cases whose ages ranged from seven months to 84 years. Sixty-nine people had faecal isolates of E.coli O157:H7. (Eight were negative using conventional methods but were positive using immunomagnetic separation (IMS)). Serological confirmation of E.coli O157:H7 infection was obtained for one child from a stored blood sample taken in the first two weeks of illness. The other child, although clinically part of the outbreak, could not be confirmed but was been included in the analysis. Epidemiological investigation revealed that over 90% of cases had consumed pasteurised milk in bottles or cartons from the same local dairy.

Results

Response Rates

Interviews took place with 69 out of the 71 (97%) cases or parents of cases, one year after the outbreak. One case which was not interviewed was a man who had moved away; the other was an elderly woman who had died. All case notes from GP and hospital inpatients were surveyed. GPs of all cases were interviewed, except for four cases who had changed GPs.

Characteristics of the cases

Most cases were children; 50% of cases were under five.

Characteristics of the illness

The illness lasted on average 6.9 weeks; 1.3 weeks per cohort cases was spent in hospital. Abdominal pain and ordinary diarrhoea were the most common combination of symptoms followed by bloody diarrhoea and pain. Eighteen cases (26%) reported suffering intermittent symptoms related to the illness 12 months later. Of those who reported that they were still suffering, 83% had had bloody diarrhoea. The mortality rate was 1.4 per hundred cases. There were 10 cases of haemolytic uraemic syndrome (HUS) and one case of thrombotic thrombocytopenia purpura (TTP). Two children were discharged on long-term dialysis, one of whom has since received a transplant. Comorbidity involving the immune system was associated with hospital admission. The relative risk of hospital admission for those with recorded co-morbidity of the immune system was 5.4 (95% CI).

Costs per case

General practice costs were made up of costs of consultation in the surgery and home visits £96; investigations £36; and treatments, which included £3 for drugs. Prescriptions were given to 19 cases. Twenty-six items were prescribed: three cases were given antispasmodics; three others were given either Calpol or paracetamol. Six cases were prescribed oral rehydration. General practice costs represented 1.5% of total costs. The paediatric outreach team visited 20 cases
at home 124 times. The average cost for other community and out-patient care was £187 for non-HUS cases, £422 for HUS cases and £1775 for TTP cases. The costs of hospitalisation, excluding the dialysis after discharge, accounted for 85% of the total costs: 95% of this was for HUS cases. The average cost of hospitalised patients was £8417. HUS cases cost on average £56 943 and the TTP case cost £16 835. Costs to cases and families were £232 for children under five; £822 for hospitalised cases; and £1434 for HUS cases. Time off work was valued as £916 per case for those of working age. Costs to carers were estimated as £811 per case. The costs of the outbreak investigation and control were collected. These included the time costs of staff involved in conducting the investigation, attending meetings and hearings in court, preparing reports and dealing with the media and the costs of consumables used in laboratory investigations. These amounted to £172 000. The costs to environmental health was £86 000; £20 000 to public health; and £18 000 for reference laboratory work. The costs were projected over 30 years for those likely to have long-term renal damage. Total projected costs were £11.9m: £168 032 per case. A sensitivity analysis of total costs was undertaken.

Discussion
The findings in this study reflect the particular characteristics of the outbreak. The estimations were based on small numbers, eg, one case of TTP. Most cases were children because the vehicle of infection was milk and because of the greater susceptibility of children to \( E. coli \) O157. Outbreaks amongst the elderly raise different issues. The outbreak caused severe illness (HUS or TTP) in 11 cases. These cases absorbed most of the costs and were most likely to report symptoms 12 months later. In generalising from this study, the distribution of children and the elderly and the proportion of severe illness should be taken into account.

References

JA Roberts, London School of Hygiene and Tropical Medicine; PA Upton, Lothian Health Board.
A case-control study of sporadic cases of *Escherichia coli* O157 infection in Scotland

M Locking

Background to the study

Since 1987, Scotland has consistently had the highest isolation rates of *E. coli* O157 in the UK. In 1998 Scotland had 4.2 cases per 100 000 population compared with 1.7 in England and Wales. Despite the identification of two of the largest described outbreaks in the world, in Lothian in 1994 and in Central Scotland in 1996, the majority of cases of *E. coli* O157 in Scotland are not recorded as part of any outbreak, and appear as sporadic cases. Since the mid-1990s, there has been a background level of between 200 and 250 sporadic cases each year, whose sources of infection are rarely identified. In 1999 (to week 46) 293 cases have been reported, compared to 188 cases the same period in 1998.

In 1992 the Chief Scientist’s Office funded a pilot descriptive study of all cases of *E. coli* O157 in Scotland, which ran for 18 months. This study found an unexpectedly high proportion of cases who had been exposed to environmental factors prior to the onset of their illness: gardening or garden play (36%); contact with farms (20%) or with farm animal by-products (17%); and recent water supply failures (12%)¹. The pilot study formed the basis for the subsequent case-control study.

Aims and objectives of the case-control study

The case-control study involved sporadic cases and aimed to determine the relative importance of the environmental exposures identified by the descriptive study, including disruptions to the domestic water supply, as risk factors for *E. coli* O157 infection.

Study design and setting

A prospective, matched case-control study was used. Up to four controls were sought for each case and were identified by either the patient’s GP or the laboratory where the clinical specimens were processed. Controls were matched for age within five years and for sex. The setting was the population of Scotland (1998 population = 5 120 000) and the study was co-ordinated by the Scottish Centre for Infection and Environmental Health (SCIEH).

Case definition

A case was defined as a person with abdominal pain or diarrhoea from whom *E. coli* O157 had been isolated on faecal culture and/or from whom serological evidence of infection with *E. coli* O157 had been demonstrated; or as a clinical case of haemolytic uraemic syndrome (HUS) with accompanying serological evidence of *E. coli* O157 infection.

Subjects were excluded from the study if they were secondary cases or if their medical histories revealed any of the following: travel abroad during the incubation period; normal residence outside Scotland; evidence of mixed infection; or a known association with an outbreak.

Case and control recruitment

Once ethical approval was obtained, case and control recruitment was carried out between October 1996 and March 1999. The study took longer than originally anticipated, mainly due to the length of time taken by the Ethical Committee process. By the end of March 1999, 183 cases had been recruited with at least one control each. There were a total of 545 matched controls. The study co-ordinator conducted a standard telephone questionnaire with both cases and controls.

Main findings of the descriptive analysis of the 183 study cases

Nearly half (44%) of the study cases were under the age of 10 and 19% were aged 60 or over. Males were significantly younger than females. Health board of residence of cases reflected previous patterns of geographical distribution in Scotland, with predominance in the North East. Diarrhoea was reported by 97%, bloody diarrhoea by 77% and 57% were admitted to hospital. Fifteen cases developed HUS all but one of whom were children.

Microbiological investigation demonstrated that 93 (51%) study cases were infected with phage type 21/28, the dominant phage type in Scotland since 1997. Molecular typing identified 59 different profiles amongst the phage type 21/28 cases. Both phenotypic and genotypic analyses confirmed that the cases were sporadic and there was no evidence of previously unidentified outbreaks.
Main findings from univariate matched analysis of cases and controls

There was no positive association with risk for any specific food, nor for disruptions to the domestic water supply. However, one apparently protective factor emerged strongly from both the univariate and multivariate analysis: drinking bottled water (P<0.0005). The exposures which had a significant positive association with risk are shown in Figure 1.

**FIGURE 1: Environmental exposures associated with significant risk of E.coli O157 infection**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds Ratio</th>
<th>P-value</th>
<th>% Cases exposed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visited farm, smallholding or zoo</td>
<td>2.34</td>
<td>&lt;0.0005</td>
<td>33.5</td>
</tr>
<tr>
<td>Contact with farm or zoo animals</td>
<td>3.34</td>
<td>&lt;0.0005</td>
<td>27.1</td>
</tr>
<tr>
<td>Having garden next to field or farm</td>
<td>2.23</td>
<td>&lt;0.0005</td>
<td>28.7</td>
</tr>
<tr>
<td>Contact with soil</td>
<td>2.80</td>
<td>0.008</td>
<td>51.0</td>
</tr>
<tr>
<td>Contact with animal faeces other than pets</td>
<td>4.02</td>
<td>&lt;0.0005</td>
<td>21.0</td>
</tr>
<tr>
<td>Likely contact with animal faeces other than pets</td>
<td>3.36</td>
<td>&lt;0.0005</td>
<td>28.4</td>
</tr>
</tbody>
</table>

In addition, within occupational categories, work with farm contact had the highest risk; within animal exposures, contact with cattle carried most risk. Children aged four to seven were most at risk of potential faecal contact. Various types of farm contact therefore showed a strong positive association with risk of E.coli O157 infection.

These findings were reinforced by the multivariate matched analysis, which showed a strong positive association with risk for contact with farm or zoo animals (P=0.061) and for contact or likely contact with faeces from animals other than pets (P=0.0670). The more complex multivariate analysis therefore indicated these exposures as carrying a strong association with risk, whatever other exposures were present.

The findings of the case-control study suggest that direct zoonotic transmission is a major route of spread. This reinforces the findings of similar studies and, as such, constitutes a positive focus for future preventive strategies.

**Project team**

Professor W J Reilly, Deputy Director (Project Leader), SCIEH; Ms M E Locking, Clinical Scientist (Study Co-ordinator), SCIEH; Dr S J O’Brien, Consultant Epidemiologist, formerly SCIEH, now PHLS Communicable Disease Surveillance Centre; Dr D Campbell, Consultant in Public Health Medicine, formerly SCIEH, now Auckland Health Care, New Zealand; Dr J Coia, Consultant Medical Microbiologist, Department of Clinical Microbiology, Western General Hospital, Edinburgh; Dr C Ramsay, Consultant Epidemiologist, formerly Lothian Public Health, now SCIEH; Professor Hugh Pennington, Medical Microbiologist, Scottish E.coli O157 Reference Laboratory, Forsterhill, Aberdeen; Dr E M Wright, Biostatistician, SCIEH; Dr Lynda Browning, Clinical Scientist, SCIEH.

**Acknowledgements**

This project was funded by the Department of Health (Project Number 247). The authors would like to thank the following for their contribution to the Project: Consultants in Public Health Medicine and their staff; Consultant and Head Microbiologists and their staff; General Practitioners and Hospital Consultants; Scottish E.coli O157 Reference Laboratory; Dr G K Adak, PHLS CDSC; Mark Myatt, Consultant Epidemiologist; Dr Gwen Allardice, Biostatistician, SCIEH.

**References**


M Locking, Scottish Centre for Infection and Environmental Health, Glasgow.
The PHLS case-control study of *Escherichia coli* O157 infection in England

GK Adak, SJ O’Brien, C Gilliam and HR Smith

**Background**

In recent years verocytotoxin-producing *Escherichia coli* O157 (VTEC O157) has emerged as a pathogen of increasing worldwide importance. There is a sharply rising trend in both the number of cases of VTEC O157 infection confirmed by LEP and the number of outbreaks reported to PHLS Communicable Disease Surveillance Centre (CDSC). PHLS data show that 87% of laboratory confirmed cases are either sporadic or involved in family outbreaks.

A study was conducted in order to investigate the epidemiology of VTEC O157 infection in England.

**Aims and Objectives**

The aim of the study was to identify and estimate the relative importance of risk factors for the acquisition of infection with VTEC O157. The objectives were:

- to identify risk factors associated with VTEC O157 infection;
- to identify factors which make implicated food vehicles more likely to cause infection;
- to investigate the role of person to person spread in the household.

**Study Design**

The study was an unmatched case-control study with GP nominated controls. Cases belonging to the following categories were excluded:

- cases who had travelled outside the UK in the five days before the onset of their symptoms;
- cases who normally reside outside England;
- cases known to be involved in general outbreaks of *E. coli* O157 infection;
- cases known to be excreting additional gastrointestinal pathogens;
- cases who had died were excluded because reliable data could not be obtained.

**Main Findings**

Data from 369 eligible cases and 511 controls were analysed. The results show that VTEC O157 infection is associated with severe morbidity in all age groups. Approximately 40% of cases were admitted to hospital. Approximately 40% of confirmed cases do not present with bloody diarrhoea.

The age and sex distribution of eligible cases is shown in Figure 1. It can be seen that infection is most common among infants.

**FIGURE 1: Age and sex distribution of eligible cases**

Infection was found to be transmitted via person-to-person spread and contact with animals as well as through the consumption of contaminated foods. The most striking specific risk factors identified were: contact with people with diarrhoea; visits to farms; travel within the UK; eating out; and exposure to recreational waters.

All of the risk factors identified are biologically plausible and reinforce previous findings. Thus the prevention of VTEC O157 infection demands the development of a range of strategies designed to tackle a variety risk factors.
Verocytotoxin-producing *Escherichia coli* (VTEC) risk factors in the farming environment

RM Chalmers, RL Salmon, J Evans, H Chart, SM Kench, TJ Coleman, D Meadows, P Morgan-Capner, P Softley, M Sallis and DRH Thomas

The study of farming populations is important in the understanding of VTEC infections, since reservoirs of infection include farmed animals, and zoonotic spread of VTEC has been identified.

The PHLS Farmworkers Study has been investigating exposure to zoonotic illness in farm workers and their families since enrolment began in 1991. Subjects at two sites (Hereford and Preston) were recruited to the study during 1991 and 1992, and subjects in a third study site (Norwich) in 1995. The cohort of 606 farmworkers has been validated as representative of people involved in agriculture in each area. During annual sampling rounds blood samples have been collected for serology, and exposure data, including the range and extent of animal contact, was collected during structured face-to-face interviews. Statistical associations between exposures and outcome were measured by calculating odds ratios (OR) and 95% Confidence Intervals (CI) for categorical variables. The extent of animal exposure was measured on a ranked ordinal scale. Ranked animal contact and the number of animals contacted were compared in positive and negative subjects by the Mann Whitney 2-sample test.

To measure seroprevalence and examine risk factors for exposure to *E.coli* O157 (using an ELISA for antibodies to the *E.coli* O157 lipopolysaccharide) a retrospective study of stored annual sera and questionnaire data collected at enrolment was undertaken. This showed that seroprevalence changed little since enrolment over three subsequent sampling rounds.

**TABLE 1: Seroprevalence of *Escherichia coli* O157 in stored sera from a cohort of farm workers in England**

<table>
<thead>
<tr>
<th>Year</th>
<th>Seroprevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hereford &amp; Preston: Enrolment</td>
<td>11/404 (2.7%)</td>
</tr>
<tr>
<td>Hereford &amp; Preston: + 1 year</td>
<td>7/385 (1.8%)</td>
</tr>
<tr>
<td>Hereford &amp; Preston: + 2 years</td>
<td>8/343 (2.3%)</td>
</tr>
<tr>
<td>Hereford &amp; Preston: + 3 years</td>
<td>3/333 (0.9%)</td>
</tr>
<tr>
<td>Norwich: Enrolment</td>
<td>0/200 (0.0%)</td>
</tr>
</tbody>
</table>

At enrolment, 11/604 (1.8%) subjects had antibodies to *E.coli* O157. All 11 had contact with sheep, compared with 63% of the cohort as a whole, and contact with sheep was identified as a risk factor for exposure to *E.coli* O157 (p=0.009). Seropositive subjects had contact with larger flocks of sheep than seronegative subjects (Mann Whitney p=0.004) and also had more frequent contact with sheep (Mann Whitney p=0.02).

A prospective study was undertaken during 1996 and 1998 to investigate faecal excretion of VTEC serotypes and seroprevalence of *E.coli O157*. A new, specific, structured questionnaire was administered with additional questions about VTEC risk factors (for example, contact with young children, food items handled and eaten). In addition to collecting blood, subjects were asked to submit a faecal specimen for testing. However, in contrast to the high annual compliance rate for blood (98%) fewer were willing to give faecal specimens (39%). Culturable faecal isolates were tested for the presence of VTEC genes by polymerase chain reaction (PCR) and serotyped, and sera were tested for *E.coli* O157 antibodies as before.

Seroprevalence during the two prospective sampling rounds was 8/487 (1.6%) and 23/459 (5%) respectively (Table 2). Crude risk factors for exposure to *E.coli* O157 included having contact with beef cattle in the last four weeks (OR=2.60, CI=1.05-6.44), having a private water supply (OR=2.62, CI=1.11-6.61), and contact with a child under five years old (OR=2.79, CI=1.14-6.83), although these were not statistically significant after adjusting for age, sex, study site and crude risk factors. Having contact with sheep was not a risk factor during the prospective study (OR=1.29, CI=0.55-3.00).
TABLE 2: Seroprevalence of Escherichia coli O157 and prevalence of VTEC serotypes in faeces in a prospective cohort study of farm workers in England

<table>
<thead>
<tr>
<th>Year</th>
<th>Seroprevalence of E.coli O157</th>
<th>Prevalence of VTEC serotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Round 1 (1996)</td>
<td>8/487 (1.6%)</td>
<td>13/208 (6.3%)</td>
</tr>
<tr>
<td>Round 2 (1998)</td>
<td>23/459 (5.0%)</td>
<td>4/167 (2.4%)</td>
</tr>
</tbody>
</table>

Verocytotoxin genes were detected in cultured E.coli isolates from 17/375 (4.5%) faecal specimens. Fewer samples contained VTEC during the winter than the rest of the year (P<0.001). VTEC serogroups isolated were O1 (from one subject), O5 (two subjects), O8 (one subject), O91 (one subject), O118 (two subjects), O128aab (two subjects), O128ac (one subject), O136 (one subject), O162 (one subject), O rough (two subjects) and undefined O serogroups (three subjects). Serogroup O157 was not isolated.

Crude risk factors for excretion of VTEC included having a private water supply (odds ratio (OR) 4.25; confidence interval (CI) 1.20-15.25); having contact with sheep in the last four weeks (OR 8.00; CI 1.02-62.77); handling raw salad (OR 9.47; CI 1.21-74.25); and handling raw poultry (OR 7.12; CI 1.54-32.97). The following remained statistically significant after adjusting for age, sex, study site, season and crude risk factors: having a private water supply (OR 6.78; CI 1.52-30.28); handling raw salad (OR 12.74; CI 1.15-140.71); and handling raw poultry (OR 11.81; CI 1.47-94.67).

Risk factors for both excretion of VTEC and exposure to E.coli O157 occur within the farming environment, particularly exposure to sheep, cattle and private water supplies. Although the same exposures were not always statistically significant in each sample round, they are all biologically plausible and it is possible that the exposures constituting a risk may change over time. For example, the prevalence of E.coli O157 in individual herds may vary. People may acquire infection directly or indirectly via contamination of private water supplies via run-off. Other sources are household members, particularly children who have been ill previously. Any approach to prevent infection would have to be multifaceted involving husbandry practices, water engineering and public education.

Acknowledgement

This study was funded by the Department of Health, grant number 254.

References

HUS Surveillance: What does it tell us about VTEC?

GK Adak, R Lynn and SJ O’Brien

Haemolytic Uraemic Syndrome (HUS) is a condition characterised by microangiopathic haemolytic anaemia, thrombocytopenia and acute renal impairment. In the late 1980s the British Paediatric Surveillance Unit (BPSU) co-ordinated a collaborative study investigating the aetiology of childhood HUS in the UK. The study demonstrated the importance of verocytotoxin-producing Escherichia coli O157 (VTEC O157) infection in the development of childhood HUS in the UK. In 1988, the final year of the study, 88 cases of VTEC O157 infection were confirmed by laboratories in the UK. By 1998 the figure had risen to 1130. It was, therefore, important to re-examine the epidemiology of HUS.

The UK Collaborative Study of Childhood Haemolytic Uraemic Syndrome began at the end of February 1997. The surveillance programme is being conducted by the BPSU, The British Association of Paediatric Nephrology (BAPN), the Public Health Laboratory Service (PHLS), the Scottish Centre for Infection and Environmental Health (SCIEH) and the Scottish Escherichia coli O157 Reference Laboratory.

Paediatricians report cases of clinically diagnosed HUS directly to the PHLS Communicable Disease Surveillance Centre (CDSC) or SCIEH by telephone at the time of diagnosis in addition to using the standard BPSU orange report card. Clinical and epidemiological data are then collected directly from paediatricians using a structured questionnaire.

Routine faeces and serum samples taken from patients are initially sent to local clinical microbiology laboratories, isolates and sera are then referred to the PHLS Laboratory of Enteric Pathogens (LEP) and the Scottish E.coli O157 Reference Laboratory for microbiological confirmation and subtyping. Information from paediatricians is then cross-matched with data from the reference laboratories.

During the first two full years of the study, data were collected for 193 clinically confirmed cases. Five children were reported to have died. Summary results are as follows: 186 cases presented with a diarrhoeal prodrome, 156 of which suffered bloody diarrhoea; stool and/or serum specimens were obtained from 185 cases and submitted for laboratory investigations and 165 of the cases of HUS were shown to have infection with VTEC O157. Half of the cases occurred in children under the age of four years (Figure 1). There was marked regional variation in the reporting of HUS (Figure 2). Some regions appeared to have more HUS cases than might have been expected from the VTEC O157 infection rate reported. Regional variations in VTEC O157 infection rates occur for a variety of reasons including the occurrence of outbreaks, local variations in screening methods policies, local differences in testing policies and real variations in incidence. If the relationship between HUS incidence and VTEC O157 infection is consistent across the country, results of our preliminary analysis suggest that many regions are under-ascertaining VTEC O157 infection.

FIGURE 1: Age and sex distribution of HUS cases

FIGURE 2: Regional distribution of HUS cases by residence

References
International surveillance of *E.coli* O157 through Enter-net

**IST Fisher, ON Gill, WJ Reilly and HR Smith, on behalf of the Enter-net participants**

**Summary**

Enter-net is an international surveillance network for human gastrointestinal infections. When the network began it involved all 15 countries of the European Union (EU), plus Switzerland and Norway; it now includes Australia, Canada, Japan and South Africa (see Figure 1). The network is funded by the European Commission (EC) and conducts international surveillance of salmonellosis and verocytotoxin producing *Escherichia coli* (VTEC) O157, including antimicrobial resistance. Enter-net is a continuation of the Salm-Net surveillance network (1994-97) which was also funded by the EC and concentrated upon harmonisation of salmonella phage-typing and the establishment of a timely international salmonella database. Through international outbreak recognition and investigation Salm-Net demonstrated that the timely exchange of information between experts in different countries can lead to effective public health action. Enter-net is continuing to extend these benefits to the prevention of *E.coli* O157 infections.

**Methods**

The overall aim of the Enter-net project is to improve understanding of the extent and evolution of anti-microbial resistance in salmonella isolates and of the distribution of VTEC O157 infections within the EU. The objectives are:

1. to collect standardised data on the anti-microbial resistance patterns of salmonellas isolated;
2. to facilitate the study of resistance mechanisms and their genetic control by arranging the collection of representative strains of multi-drug resistant (MDR) salmonellas and co-ordinating the required research work between specialised centres and, where available, compare the resistances of animal isolates;
3. to extend the typing of VTEC for surveillance purposes by:
   a) extending the availability of phage-typing for *E.coli* O157;
   b) using poly- and mono-valent anti-sera to identify common non-O157 serogroups;
4. to pilot an international quality assessment scheme for laboratory methods used in the identification/typing of VTEC;
5. to establish a core set of data items to accompany, where possible, each laboratory typed VTEC isolate;
6. to create an international database of VTEC isolates which is updated regularly and is readily available to each participating team;
7. to detect clusters of VTEC isolate types in time, place and person and to bring such clusters to the attention of collaborators rapidly;
8. to support the above objectives by continuing the existing Salm-Net surveillance system consisting of regular, frequent data exchange on salmonellas

**International outbreaks recognised**

Objective seven of the above is to recognise international outbreaks of enteric infections and there have been many recognised and investigated since the inception of Enter/Salm-net. Table 1 lists these.
TABLE 1: International outbreaks which were recognised and investigated by Enter/Salm-net

<table>
<thead>
<tr>
<th>Outbreak</th>
<th>No of cases</th>
<th>Countries with cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.paratyphi B²</td>
<td>300+</td>
<td>Denmark, England &amp; Wales, Finland, Germany, Ireland, Norway, Sweden and Switzerland</td>
</tr>
<tr>
<td>S.newport ³</td>
<td>100+</td>
<td>England &amp; Wales, Finland</td>
</tr>
<tr>
<td>S.livingstone ⁴</td>
<td>100+</td>
<td>Austria, the Czech Republic, Denmark, England &amp; Wales, Finland, France, Germany, Netherland, Norway and Sweden.</td>
</tr>
<tr>
<td>E.coli O157/HUS ⁵</td>
<td>15</td>
<td>Denmark, England &amp; Wales, Finland Sweden</td>
</tr>
<tr>
<td>S.anatum ⁶</td>
<td>19</td>
<td>England &amp; Wales, France, Scotland</td>
</tr>
<tr>
<td>S.agona ⁷</td>
<td>4000+</td>
<td>Canada, England &amp; Wales, Israel, USA</td>
</tr>
<tr>
<td>S.dublin ⁸</td>
<td>30+</td>
<td>France, Switzerland</td>
</tr>
<tr>
<td>S.stanley ³</td>
<td>100+</td>
<td>Finland, USA</td>
</tr>
<tr>
<td>S.tosamanga ¹⁰</td>
<td>28</td>
<td>Eire, England &amp; Wales, France, Germany, Sweden, Switzerland</td>
</tr>
<tr>
<td>Shigella sonnei¹¹</td>
<td>100+</td>
<td>England &amp; Wales, Germany, Norway, Scotland, Sweden</td>
</tr>
</tbody>
</table>

Conclusion

Enter-net has set new standards for the surveillance of VTEC O157 and salmonella infections, including antimicrobial resistance. It is a model of how focused, infection-specific international surveillance involving the key public health professionals can function effectively. Harmonisation of public health methodologies and protocols for the surveillance of enteric disease has facilitated the prevention and control of foodborne outbreaks. The timely exchange of reliable data and information between authoritative sources is critical to early outbreak recognition and subsequent comprehensive investigation. The effectiveness and efficiency of measures used to control international foodborne outbreaks are closely related to the extent of collaborative investigations. Regular meetings of leading reference microbiologists and epidemiologists facilitate the development of a common approach to sub-typing organisms which is essential for international surveillance. Enter-net represents an example of how international surveillance systems can be developed to meet the demands of the modern world well into the 21st Century.

References:

1. IST Fisher on behalf of the Enter-net participants. The Enter-net international surveillance network - how it works. Eurosurv 1999; 4: 52-55.
Genetic heterogeneity of *Escherichia coli* O157 in Scotland and its utility in strain typing

F Thomson-Carter, L Allison and P Carter

*Escherichia coli* O157 is a significant human pathogen causing both sporadic cases and outbreaks of infection. In the recent past Scotland has experienced several major outbreaks of *E. coli* O157 infection in addition to a relatively increased incidence of sporadic infection compared with the remainder of the UK.

Since 1992 all *E. coli* O157 isolated in Scotland have been typed at the Scottish Reference Laboratory for *E. coli* O157 and Campylobacter using a hierarchy of phenotypic and genotypic methods. The prime objective in typing bacteria is to characterise them definitively and, thus, distinguish probable outbreak-associated isolates from apparent sporadic isolates. In pursuing this objective the population structure of the pathogen must be considered: *E. coli* and its constituent sub-specific groups is thought to be clonally derived (ie, descended from a common ancestor on the basis of multilocus enzyme electrophoretic analyses). The working definition of a clone used by the Scottish Reference Laboratory is that proposed by Orskov and Orskov: “... bacteria isolated independently from different sources, in different locations, and perhaps at different times, but showing so many identical phenotypic and genetic traits that the most likely explanation for this identity is a common origin”.

The identification and tracking of clones from human and human infection-associated sources (food, animal, environmental) within Scotland is achieved using a hierarchy of phenotypic and genotypic techniques. Phenotypic methods (ie, those relating to expressed characteristics of the bacterium) include biotyping, serotyping and bacteriophage typing. Genotypic methods or those based on the genetic content of the bacterium include polymerase chain reaction (PCR) assays, analyses of various restriction fragment length polymorphisms (RFLPs), pulsed-field gel electrophoresis (PFGE), amplified fragment length polymorphism (AFLP) and gene sequencing. All these methods have advantages and disadvantages for bacterial typing but genotypic methods, although sometimes more technically complex, are generally considered more robust and yield more consistent data.

During the period 1 April 1998 to 31 March 1999, 66.2% of all *E. coli* O157 isolates received by the Reference Laboratory typed as phage type 21/28; 14.5% of all *E. coli* O157 isolates received typed as phage type 2. The majority (80.7%) of all *E. coli* O157 isolated in Scotland, therefore were assigned to one of only two distinct phage types. However, PFGE analyses of these isolates using XbaI to cleave genomic DNA demonstrated that more than thirty PT 21/28 and ten PT 2 PFGE sub-types respectively could be resolved, thus providing greater definition and optimal characterisation of individual isolates.

Variant PFGE sub-types can be identified in all categories of isolate referred: human; animal; food; and environmental. They have been isolated over a period of approximately five years from locations throughout Scotland; certain of the variant PFGE subtypes have been identified only in humans to date. These results indicate that there is sufficient genetic heterogeneity within the Scottish *E. coli* O157 population to exploit for epidemiological purposes. Sufficient variation exists in the PFGE macrorestriction profiles resolved to enable tracking of particular isolate sub-types and, crucially, to differentiate outbreak-associated isolates from the generally high background of apparently sporadic isolates in Scotland.

The day-to-day work of the Reference Laboratory has demonstrated the utility of this characteristic in typing of *E. coli* O157. Seven discrete outbreaks occurred in Scotland during the twelve-month period April 1994 to March 1995, including the largest milk-borne outbreak to date world-wide. Various vehicles of infection were identified and there were 144 confirmed cases in total. All isolates associated with the outbreaks were subjected to detailed sub-typing: phage typing, carriage of verotoxicin genes, and PFGE. The outbreak strains were of three different phage types (2, 4 and 21/28). Those of phage type 2 and 21/28 were VT1-/VT2+, those of phage type 4 were VT1+/VT2+. Real-time PFGE analyses demonstrated that, within each of the seven outbreak groups, the macrorestriction profiles observed were indistinguishable, whereas profiles obtained for sporadic isolates were not.

Four of the seven outbreaks studied in this period were apparently caused by recurrent clonal types of *E. coli* O157 (ie, two previously identified PFGE macrorestriction profiles were resolved). The remaining three outbreak groups were apparently sporadic, having unique PFGE profiles.

Genotyping of *E. coli* O157 has been further developed in the Reference Laboratory to optimise discrimination between and among isolates, particularly those which are closely related: modulation of PFGE run parameters to resolve larger
or smaller DNA fragments; application of a hierarchy of restriction endonucleases; application of other molecular methods which index the bacterial genome differently from PFGE. Using this type of approach it is possible to identify consistent, reproducible variation in the genomes of Scottish *E.coli* O157 isolates. The Central Scotland Outbreak (1996) group of isolates for example, can be differentiated from both the West Lothian (1994) and Highland (1994) outbreak groups\(^3\). The accurate molecular description of the Central Scotland outbreak strain has enabled its widespread dispersal across Scotland to be monitored: from July 1992 to November 1996 this particular sub-type had been seen on only one previous occasion. Since November 1996 it has been isolated during nine separate incidents at locations throughout Scotland.

Interpretation of PFGE and other genotypic data in epidemiological investigation is not routine. In efforts to establish guidelines for PFGE data it has been postulated that macrorestriction profiles obtained for a range of bacterial genera and differing by as many as six fragments from that of a known outbreak isolate may yet be outbreak-associated\(^4\). The work of the Reference Laboratory has shown that for *E.coli* O157, with only a few exceptions, all isolates associated with a particular outbreak exhibit indistinguishable PFGE profiles irrespective of their source (human, animal, food or environmental).

The degree of genomic diversity identified by PFGE is greater than that predicted by population genetic studies on selectively neutral alleles using multilocus enzyme electrophoresis, which demonstrate that *E.coli* O157 belongs to a single clone. The high degree of diversity apparent raises a number of major questions concerning genetic polymorphisms in *E.coli* O157, in particular their stability and distribution, which impact directly on development and validation of typing methods and interpretation of the data generated. The recent identification of the same novel DNA region in both *E.coli* O157 isolated from diverse sources and bovine commensal *E.coli* isolates, but its absence from human commensal *E.coli* isolates only serves to demonstrate that the dynamics of the natural *E.coli* O157 population in Scotland have yet to be fully elucidated.

**Acknowledgements**

The expertise of all Reference Laboratory staff and the co-operation of colleagues in submission of isolates are gratefully acknowledged. The Reference Laboratory is funded by NSD.

**References**

Verocytotoxin-producing Escherichia coli O157 in England and Wales: update on laboratory studies

HR Smith, GA Willshaw, T Cheasty and SJ O’Brien

Human infections caused by verocytotoxin-producing Escherichia coli O157 (VTEC O157) have continued to increase in England and Wales as well as the rest of the UK. Part of this increase has resulted from improved methods for detection as well as increased testing and reporting, particularly following the very large outbreak in Central Scotland in 1996. Laboratory surveillance plays a vital role in the confirmation of cases and typing of isolates for epidemiological investigations.

During the period 1995 to 1998 the annual totals of VTEC O157 isolates for England and Wales were 792, 660, 1087 and 890, respectively. For the first nine months of 1999 the Laboratory of Enteric Pathogens (LEP) at the Public Health Laboratory Service (PHLS) has confirmed 912 isolates compared with totals of 685 and 862 for the corresponding period in 1998 and 1997 respectively. For the years 1995 to 1998 most VTEC O157 strains were VT2 only (76%) with 23.3% encoding both VT1 and VT2 and the remaining 0.7% carrying only VT1 genes. The level of antimicrobial resistance in the VTEC O157 isolates has increased, but resistance to four or more drugs is rare. From 1995 to 1998 between 17.4% and 22.8% of isolates were resistant to at least one antimicrobial agent, with the most common resistance patterns being SSu, SSuT and SuT (S: streptomycin; Su: sulphathiazole, T: tetracyclines).

Incidence of infection continued to be highest in children aged one to four years with the seasonal peak in July, August and September. There were marked regional variations in incidence and some of these were significantly affected by outbreaks. For example, in 1997 the rate in Trent was 3.25 per 100 000 and there were six general outbreaks. In 1998 the regions with the highest incidences were Northern and Yorkshire (2.45 per 100 000 population), Wales (2.15 per 100 000) and South and West (2.1 per 100 000). The lowest incidences were in South Thames (1.29 per 100 000) and North Thames (1.3 per 100 000). A small but increasing proportion of VTEC O157 infections reported in England and Wales was linked to foreign travel. The percentages of the total associated with recent foreign travel were 5.2 (1995), 9.1 (1996), 10.8 (1997) and 10.1 (1998).

The majority of human infections with VTEC O157 appear to be sporadic and only about 12% of the total cases for 1995 to 1998 were outbreak related. However, there were at least 67 general outbreaks of infection during this period and there have been sixteen outbreaks in the first ten months of 1999. Over 80% of the outbreaks were attributed to strains of phage type 2 (PT2), PT8 and PT21/28. Most outbreaks were community based, with several linked to premises such as restaurants, catering outlets and butchers’ shops. Outbreaks also occurred in institutions such as nurseries and residential homes for the elderly. Direct or indirect animal contact was an important route of transmission, with nine of the outbreaks between 1996 and 1998 linked to visits to “open” farms or other farms. In the last two years there have been several outbreaks and incidents resulting from the consumption of contaminated dairy products. These have included raw milk, milk contaminated as a result of pasteurisation failure or contaminated after pasteurisation, as well as cheese or cream made from raw milk.

Typing of confirmed VTEC O157 isolates has provided much valuable information for epidemiological investigations. The combined use of phage typing, VT typing and pulsed-field gel electrophoresis (PFGE) gives very good discrimination. In 1998 the isolates belonged to 20 different phage types. PT2 continued to be predominant, but declined from 54% in 1995 to 31% in 1998. The other most common types in 1998 were PT8 (18%) PT21/28 (16%) and PT4 (8%) and PT32 (8%). The importance of PT21/28 has continued in 1999: it is currently the most common type and has been the “outbreak” strain on seven occasions. Within these common phage types, VT gene subtyping and PFGE has provided further subdivision and this has been essential in outbreak investigations. For example, in eleven outbreaks caused by PT8 strains, two strains were different phenotypically, whereas all eleven outbreak strains were distinct by PFGE of XbaI restriction fragments. This approach was important in distinguishing cases of sporadic infection from those that were part of outbreaks and provided further evidence for the association of food vehicles or animals with human disease.

Enhanced surveillance, both laboratory and epidemiological, is required in order to provide the information necessary for introduction of control measures.

Improved methods of *E.coli* O157 detection in food

ID Ogden, M MacRae and N Hepburn

To optimise the detection of *E.coli* O157 from food, the protocol must utilise and take into account the a number of factors including:

- low numbers of target bacteria, because of the low infectious dose of *E.coli* O157;
- detection of injured cells, caused by food processing and storage;
- cocktail of strains, to minimise the influence of atypical single strains;
- number of different foods, validated in those foods most often associated with *E.coli* O157;
- high number of competitors, to detect the target in the presence of high numbers of healthy non-*E.coli* O157 bacteria.

Due to the difficulty in obtaining large volumes of naturally contaminated food, this work has utilised spiked samples which enable the points listed above to be complied with fully. Validation was carried out in naturally contaminated cheese and mince samples.

It is widely accepted that immuno-magnetic separation (IMS) methods have improved the recovery rates of *E.coli* O157 from foods, and these are now used throughout the world in routine testing laboratories. However, initial protocols were established for the isolation of *E.coli* O157 from faecal samples and were found to be unsuitable for some food types.

IMS has several integral components (see below), each having an important bearing on the success of target recovery. The work in this laboratory has sought to optimise all aspects of the IMS method, eg:

- enrichment broth;
- temperature;
- time;
- volume;
- selective agars.

To ensure replicate food sample composition, mince (100g) was spiked with low numbers (10²/g) of *E.coli* O157 cocktail and subjected to a series of freeze/thaw cycles which resulted in a reduction in level to <1/g (enumerated by MPN) of stressed bacteria. This was added to 100g of unfrozen mince containing high (10⁵/g) of background microflora and homogenised in PBS. 50ml volumes were then added to double strength enrichment broth under test, thus ensuring exact replication between samples. The types of enrichment broth and their performance are shown in Table 1. IMS beads were plated in duplicate onto CTSMAC incubated at 37°C.

<table>
<thead>
<tr>
<th>Enrichment medium</th>
<th>37°C</th>
<th>40°C</th>
<th>42°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPW-V pH 7.0</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>BPW-V pH 6.0</td>
<td>+</td>
<td>NT</td>
<td>+</td>
</tr>
<tr>
<td>BPW VCC</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>BPW-V + ¼ C + C</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>m TSB + N</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>EC broth + N</td>
<td>+/-</td>
<td>NT</td>
<td>+</td>
</tr>
<tr>
<td>Peptone tween</td>
<td>+</td>
<td>NT</td>
<td>+</td>
</tr>
</tbody>
</table>

The composition and temperature of enrichment was shown to have a dramatic effect and three broths were chosen for further validations: BPW-V pH 7.0 at 42°C; BPW VCC at 37°C; and m TSB + N at 42°C. Three foods were used in addition to minced beef: cheese; apple juice; and pepperoni. Stressed cocktails were added to apple juice from survivors in apple juice with a pH 3.9, stored at 4°C for ten days. Stressed cultures were added to sliced pepperoni from a cocktail derived from a high salt (13.5% w/v), low pH (4.9), low temperature (5°C) broth over a 10-day period. Cheese was surface spread with 0.5ml of the *E.coli* O157 cocktail containing approximately 10⁸/ml and stored at 4°C for two days prior to analysis, thus simulating post processing contamination. The results are shown in Table 2.
The conclusion we draw from these studies is that buffered peptone water supplemented with vancomycin (8mg/l) incubated at 42°C gives the best recovery with minimum background flora. This is particularly important where stressed cells are encountered and where this broth gives greater recovery than m TSB + N 42°C, the proposed ISO medium. This could be due to the presence of bile salts in the mTSB inhibiting physiologically damaged *E.coli* O157.

A comparison of different selective agars was carried out, all incubated at 37°C. Results are shown in Table 3. Further validation was performed using naturally contaminated foods as shown in Table 4.

### TABLE 2: Validation of IMS protocols with other foods

<table>
<thead>
<tr>
<th>Enrichment medium</th>
<th>Cheese</th>
<th>Apple juice</th>
<th>Pepperoni</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>stressed</td>
<td>unstressed</td>
<td>stressed</td>
</tr>
<tr>
<td>BPW - V 42°C</td>
<td>+</td>
<td>+ + +</td>
<td>+ +</td>
</tr>
<tr>
<td>BPW -VCC 37°C</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>m TSB + N 42°C</td>
<td>+ +</td>
<td>+</td>
<td>+ +</td>
</tr>
</tbody>
</table>

### TABLE 3: Comparison of *E.coli* O157 agars

<table>
<thead>
<tr>
<th>Media</th>
<th>Rating</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTSMAC</td>
<td>+ + + +</td>
</tr>
<tr>
<td>SMAC</td>
<td>+</td>
</tr>
<tr>
<td>SMAC + 1/3 CT</td>
<td>+</td>
</tr>
<tr>
<td>SMAC + 2/3 CT</td>
<td>+</td>
</tr>
<tr>
<td>Chromagar EE220</td>
<td>+ + + +</td>
</tr>
<tr>
<td>SD39</td>
<td>-</td>
</tr>
<tr>
<td>Rainbow</td>
<td>+ + + +</td>
</tr>
</tbody>
</table>

### TABLE 4: Numbers of foods positive for *E.coli* O157 by three enrichment procedures

<table>
<thead>
<tr>
<th>Enrichment</th>
<th>Cheese No. positive</th>
<th>Mince No. positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPW – V 42°C</td>
<td>3/6</td>
<td>1/2</td>
</tr>
<tr>
<td>BPW VCC 37°C</td>
<td>0/6</td>
<td>0/2</td>
</tr>
<tr>
<td>m TSB-N 42°C</td>
<td>1/6</td>
<td>0/2</td>
</tr>
</tbody>
</table>

Optimal recovery was seen on Rainbow agar (Biolog) and poorest recovery was shown by SD39 (Quality Life Sciences *E.coli* O157 isolation medium) where target bacteria were often overgrown with background growth. We routinely use two plates to plate the IMS beads, one based on sorbitol MacConkey and the other based on chromogenic reactions. This allows for those *E.coli* O157 that are sorbitol positive and sensitive to cefixime and tellurite to be detected on an alternative medium. This laboratory uses CTSMAC, which most usually gives very good recovery with little background, and Chromagar EE220 which, although a little poorer than Rainbow agar, is somewhat cheaper.

This work has demonstrated that for optimal recovery of damaged *E.coli* O157 in the presence of high numbers of healthy background bacteria, buffered peptone water + vancomycin (8mg/l) incubated at 42°C followed by plating the beads on CTSMAC and Chromagar EE220 gives greatest recovery.

Problems with fatty foods affecting IMS bead recovery have been reported. Treatment of enriched samples with equal volumes of n-heptane has been shown to improve bead recovery while not affecting the viability of *E.coli* O157.

Routine IMS protocols test 1ml enrichment volumes. Preliminary work has shown that increasing this to 10ml or 50ml, while maintaining the bead volume at 0.02ml, increases the recovery of *E.coli* O157. This is despite the lack of custom made apparatus for increased volumes. Testing 10ml volumes on foods spiked with low numbers (<1/g, both stressed and unstressed) *E.coli* O157 has allowed us to reduce the standard enrichment incubation time from 6h to 4h. Improved bead recoveries from 50ml volumes suggest that this time could be reduced further.

Alternative methods to detect *E.coli* O157 with increased sensitivity are under test using ELISA type tests incorporating O157 antibodies labelled with Russel’s Viper Venom. This activates the blood clotting cascade reaction converting a small amount of fibrinogen to a large amount of fibrin. Fibrin was optically detected by the production of clots in microtitre pates. Detection levels of 10^3/ml have been observed in preliminary tests. When coupled to optimum enrichment from large IMS volumes, detection of low numbers of stressed *E.coli* O157 within a working day is possible.

Our thanks to the Scottish Executive Rural Affairs Department (SERAD) who have fully supported this work.

ID Ogden, M MacRae, N Hepburn, Applied Food Microbiology Group, Department of Medical Microbiology, University of Aberdeen.
Survey of the prevalence of VTEC O157 in raw meats, raw cow’s milk and raw milk cheeses in South-east Scotland

JE Coia and MF Hanson

Infections caused by verocytotoxigenic *Escherichia coli* O157 (VTEC O157) have emerged as a major public health concern in North America and Europe. In Britain the highest recorded rates of infection in recent years have been in Scotland, where 506 cases (9.8/100,000) were recorded in 1996. Although meat and dairy products have been implicated in many outbreaks, the paucity of isolations from food has meant that much remains unclear about the epidemiology of the infection. Lack of suitable methods of sufficient sensitivity for routine detection of the organism in foodstuffs have frustrated attempts to further define these associations. The technique of immunomagnetic separation (IMS) has revolutionised the laboratory’s ability to isolate the organism. In the Department of Clinical Microbiology at the Western General Hospital, Edinburgh, the technique was successfully employed in the investigation of a large milkborne outbreak in West Lothian. Given high local rates of infection, epidemiological data incriminating bovine products and availability of IMS methodology, the Department of Health funded this laboratory to test retail meats, unpasteurised milks and unpasteurised milk products from South-east Scotland for the presence of VTEC O157.

Immunomagnetic separation (IMS) was used to examine 2429 samples of foodstuffs over a 2 year period commencing April 1997. Specimens were collected by local authority Environmental Health Officers (EHOs) from City of Edinburgh, West Lothian, Midlothian, East Lothian and Scottish Borders and comprised 1190 raw meats (Beef and lamb products were sampled in the ratio 80:20), 500 raw milks and 739 raw milk cheeses. Meat and cheese samples were purchased from retail premises in south-east Scotland; raw milks were obtained directly from farms. In addition, total *E.coli* counts were performed on milk and cheese samples, and the pH of cheese specimens measured. A representative sample of each cheese type, and all of those with high levels of *E.coli*, had water activity (Aw) measured.

The study demonstrated a very low prevalence of contamination with VTEC O157 in raw retail beef products (0.24%). *E. coli* O157 was not detected in any other samples. Control studies with artificially inoculated foodstuffs demonstrated a sensitivity of detection of <5 organisms 25 g⁻¹.

With regard to total *E.coli* counts, the standard of raw milks was good, with almost 98% having less than 100 per ml present. The cheese results were less encouraging. Almost 10% of samples would have been classified as having counts in either the undesirable or unsatisfactory/potentially hazardous categories under the latest guidelines.

These findings contrast with results of some studies elsewhere in the UK, and suggest other sources of infection may be important in explaining the high rates of infection with this organism in South-east Scotland. Although foodborne contamination may be a catastrophic event, as evidenced by the West Lothian dairy and Central Scotland meatborne outbreaks, such contamination may be a relatively uncommon event.

Acknowledgements

This work was funded by the Department of Health (Project Code DH250). The authors would like to thank:

The Scottish *E.coli* O157 Reference Laboratory; The Scottish Office Agriculture & Fisheries Department; The Scottish Centre for Infection and Environmental Health; Dr. Paul Cook, Department of Health; City of Edinburgh Council; West Lothian Council; Midlothian Council; East Lothian Council; Scottish Borders Council; Mr. Nicholas Steers; and Mrs. Yvonne Johnston.

References


JE Coia, MF Hanson, Western General Hospital, Edinburgh.
Escherichia coli O157:H7 and dairy products in the North-west of England

HC Aird, FJ Bolton and PA Wright

The Public Health Laboratory at Preston is part of the PHLS North West Group. The Laboratory has a special interest in E.coli O157 and VTEC and is involved in a number of projects based on surveillance and detection of these organisms in cases of human disease and in food.

Since 1991 in the Lancashire and South Cumbria areas there have been six notable outbreaks of infection associated with consumption of dairy products including yoghurt, milk and cheese (Table 1).

**TABLE 1: Outbreaks of E.coli O157 infection associated with dairy products in the North west of England 1991-1999**

<table>
<thead>
<tr>
<th>Year</th>
<th>No. cases (HUS)</th>
<th>Suspected Food</th>
<th>Isolation from Suspected Food</th>
<th>Phage Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1991</td>
<td>16(5)</td>
<td>Yoghurt1</td>
<td>no</td>
<td>49</td>
</tr>
<tr>
<td>1996</td>
<td>9(1)</td>
<td>Milk2</td>
<td>no</td>
<td>2</td>
</tr>
<tr>
<td>1997</td>
<td>5</td>
<td>Unpasteurised Cheese</td>
<td>yes</td>
<td>8</td>
</tr>
<tr>
<td>1998</td>
<td>3</td>
<td>Unpasteurised Milk</td>
<td>yes</td>
<td>8</td>
</tr>
<tr>
<td>1999</td>
<td>11(3)</td>
<td>Milk3</td>
<td>no</td>
<td>4</td>
</tr>
<tr>
<td>1999</td>
<td>4</td>
<td>Cheese4</td>
<td>no</td>
<td>4</td>
</tr>
</tbody>
</table>

1,2,3,4 see References

The first dairy associated outbreak identified in 1991 was associated with locally produced live yoghurt. Sixteen cases were identified, five of which developed HUS. This was the first outbreak of O157:H7 to be associated with yoghurt a product which was originally thought to be safe due to its acidity.

In 1996 there was an outbreak in East Lancashire associated with milk which resulted in nine cases of infection and one HUS case this was thought to have occurred due to pasteurisation failure.

In 1997 the second outbreak in the UK, due to contaminated unpasteurised cheese, occurred in Lancashire associated with a local cheese producer. In collaboration with a number of Local Authorities, CDSC North West and LEP five cases were identified: two had a direct link with the cheese in question; two had an indirect link; and one case could not be linked but was included because he lived in the area and the strain isolated from him was identical to that isolated from the cheese, the other human cases, and from the dairy herd that supplied milk for the cheese.

In 1998 a small outbreak associated with unpasteurised milk was identified: the causative strain was isolated from the dairy herd; the milk; a milk sock; and all three cases.

More recently we have experience a large outbreak associated with pasteurised milk from a farm with an on-farm pasteuriser - this affected a number of nurseries in the North Preston area. The causative strain (which was of PT 4) was isolated from the dairy herd but was not isolated from the milk. Further investigation suggested that post pasteurisation contamination may have been the cause of this outbreak.

Another interesting outbreak occurred in Lancashire linked with a exclusive restaurant: this may have been due to homemade cheese. Four cases occurred, one of which was a food handler.

Outbreaks have occurred due to consumption of unpasteurised products but also as a result of pasteurisation failure or post pasteurisation contamination.

Dairy products are a significant and increasing cause of outbreaks in the North west of England. Extensive investigations involving many agencies including Local Authorities, Public Health Laboratories, regional and national epidemiology centres and reference facilities are essential to investigate and draw these outbreaks to satisfactory conclusions.

To minimise the risk from dairy products stricter controls on small on-farm dairies, which are common in the North West of England, is necessary. Introduction of HACCP principals at each step of production, along with prohibition of the sale of unpasteurised dairy products, will be essential to minimise exposure of the population to this potentially life threatening organism.

References


H Aird, FJ Bolton, PA Wright, PHLS North West, Preston.
Survival of *Escherichia coli* 0157 in water and organic wastes on land: the potential to contaminate untreated private water supplies

CW Keevil

Verocytotoxic strains of *Escherichia coli* (VTEC) are responsible for food-borne and water-borne infection, predominantly via contaminated cattle faeces. Consequently, VTEC must adapt from an anaerobic, nutrient rich, warm environment *in vivo* to more stressful environments *in vitro*. Food and food processing environments present a wide variety of environmental stresses which successful water-borne or food-borne pathogens must adapt to, including extremes of heat, pH and oxygen concentration. Indeed, *E. coli* O157 adapts well between aerobic and anaerobic environments and produces slightly more of the verocytotoxins (VT1 and VT2) during oxygen-limited or anaerobic growth, compared to aerobic growth. It also becomes more adhesive to human enterocytes at lower oxygen concentrations, perhaps due to production of colanic acid exopolysaccharide, which may provide additional environmental protection against desiccation\(^2\). Several of the large O157 outbreaks in Japan in 1996 were associated with the consumption of contaminated radish sprouts: of note, the strains involved were subsequently shown to be particularly resistant to desiccation and, therefore, more able to survive for long periods on the sprouts before consumption\(^3\).

Contaminated animal and human faeces can enter the food chain and water cycle by several routes, including direct defaecation by grazing livestock and wild animals to land, and the beneficial application of treated sewage sludge and animal wastes, manures and slurries to land. If the latter are Improperly stored and/or treated, then there is a potential risk for transfer of *E. coli* O157 directly to land that may be used to grow crops. Over 500 000 tonnes dry weight of sludge are recycled to land in the UK each year, compared to over 80 million tonnes wet weight of cattle, sheep and pig wastes. *E. coli* O157 contamination has been reported of fruit (by flies carrying faeces on their legs), windfall apples (in orchards grazed by cattle) and vegetables (due to application of manure). The first O157 outbreak reported in the UK involved cross contamination in the kitchen with manure-contaminated potatoes\(^4\). Furthermore, eight people were infected with *E. coli* O157 (PT2 VT2) at the 1997 Glastonbury outdoor music festival which was affected by heavy rainfall. This was perhaps due to ingestion of mud contaminated with cattle faeces containing the pathogen\(^2\). The cattle herd concerned contained the same type strain and had not grazed on the land for many weeks, indicating the potential for VTEC to survive on land for prolonged periods.

Waterborne outbreaks of *E. coli* O157 have been described, including 20 000 cases in Swaziland in 1992 and 243 cases in Cabool, Missouri in 1989, the latter resulting in four deaths\(^5\). This has caused concern that the pathogen may survive long enough in faeces-contaminated soil to wash into surface waters where it could pollute potable water supplies, recreational waters or water used for crop irrigation. Work at the Centre for Applied Microbiology and Research (CAMR) has utilised laboratory-based model ecosystems to show that verocytotoxic *E. coli* O157 is a robust pathogen with the potential for long periods in environments, including cattle faeces, slurry, soil and river water\(^6\). Various strains, including human isolate PS14 (VT1 VT2), appeared to survive many weeks in cattle faeces and in soil microcosms (Figure. 1). Several-fold greater numbers were recovered on the less stressful CHROMagar medium, compared to SMAC or CT-SMAC agar. This might suggest the presence of sub-lethally damaged cells surviving in the soil environment which require improved resuscitation methods for detection. Current work for the UK water industry, MAFF and other government departments is developing novel quantitative resuscitation protocols to address problems of detecting stressed pathogens in organic wastes. *E. coli* O157 also persisted in unsterilised river water with an autochthonous microbial population for over three weeks, indicating that it is successful in resisting competing bacterial species and eukaryotic predators. The pathogen has been reported to survive many months in cattle trough sediments, and also intracellularly within *Acanthamoeba* spp., which may provide an important environmental reservoir for survival in soil and water\(^5\).

Bacteria carrying the VT2 gene, and *E. coli* O157: H7 itself, have been found to occur in significant numbers in rivers\(^7\). Moreover, outbreaks have been described involving swimming in recreational lakes or pools in Europe and the USA\(^6, 17\). Recently, it has become clear from European and American studies that private and/or untreated drinking water supplies represent a significant risk for transmission of *E. coli* O157 from faecal ingress\(^6, 15\). A private water supply is defined as any water supply that is not provided by a statutorily appointed water company\(^16\). Local authorities have a statutory obligation to monitor private water supplies, and the specified frequency of sampling varies between two samples a month to one sample every five years, depending upon consumption rates. Private water supplies are divided into two categories: those wholly for domestic purposes; and those supplying premises such as hospitals, residential homes, holiday sites and food preparation premises. A PHLS survey of private water supplies found that 13-18% of private water supplies were positive for *E. coli* (non-pathogenic indicator strains) between January to June.
1997 and 40-42% were positive between July to December 1996¹. Following this survey, an outbreak involving 14 cases in South-west England was associated with consumption of private water: *E.coli* O157 PT2 VT2 was identified from the patients and was also isolated from a house tap¹. Scotland has suffered several private water-borne outbreaks, including five cases of *E.coli* O157 PT2 in Freuchie, Fife in 1995. In a recent outbreak in Applecross, West Scotland, six tourists were infected with PT21/28 VT2 and this strain was isolated from two different sources supplying private water¹. In the USA, over 60 people were infected during an outbreak of *E.coli* O157 involving a municipal water supply in Alpine, Wyoming in 1998, and 21 patients required hospitalisation. But by far the largest private water outbreak occurred at the Washington County Fairground near Albany, New York, in 1999. Over 1000 people were infected, 65 were hospitalised and two died, although some cases of *Campylobacter* infection were also involved¹. Nine children were very poorly due to developing haemolytic uraemic syndrome and required long-term dialysis. All of those infected drank water contaminated with manure that had seeped into a non-chlorinated well from a nearby dairy barn after a torrential downpour.

Current work at CAMR is investigating survival of *E.coli* O157:H7 as dried deposits on various work surface materials, and in the biofilms on metal or plastic pipes supplying potable water¹. These latter studies, similar to previous work with non-toxigenic coliforms¹, show that the pathogen was able to persist for many weeks in chemostat model systems receiving a continuous flow of a soft potable water at 10°, 20° and 40°C. This water type is typical of an upland catchment supply found in Scotland and elsewhere. The lower temperatures are representative of water supplied to buildings in moderate or hot seasons; the higher temperature reflects poorly heated hot water supplies or those with poorly insulated pipes. Perhaps more importantly, however, *E.coli* O157 was able to colonise biofilms of high species diversity and persist for several weeks at the lower temperatures (Table 1).

Significantly, higher numbers were detected in the biofilms on plastic pipework compared to copper or stainless steel. Similar results have now been obtained using moderately hard and hard potable waters. This suggests that the type of plumbing material used for supplying poorly treated water to the home or factory is important, and that use of copper pipe might confer some public health benefit. These studies support the available epidemiological evidence that private water supplies with inadequate disinfection treatment may be at particular risk of harbouring and transmitting *E.coli* O157. It may be necessary to reconsider the current legislation for maintaining private water supplies to avoid the transmission of potential waterborne pathogens.

**TABLE 1: E.coli O157 (human isolate PS 14) survival in potable water biofilms formed on different materials at temperatures typical of cold and warm water supplies**

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Glass</th>
<th>Copper</th>
<th>Stainless Steel</th>
<th>Polybutylene</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+/—</td>
</tr>
<tr>
<td>20</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>40</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>

+/- denotes little or no colonisation; + denotes light colonisation; ++ denotes good colonisation; +++ denotes heavy colonisation

**References**

Dispersal of *E. coli* O157 in soil and contamination of water

JM Hall (now Ritchie), Y Beaton, LA Glover and K Killham

The environment may act as a reservoir for human pathogens such as *E. coli* O157; therefore, there is a need for a greater understanding of the mechanisms and factors which control pathogen population dynamics in soils. *E. coli* O157 exists harmlessly within the guts of bovine animals, and is deposited onto the soil surface in faeces (either from individual animals or through slurry spreading onto land). There are very few studies investigating the persistence of *E. coli* O157 in soil; however, Maule found that *E. coli* O157 was capable for surviving for a number of weeks. Displacement of *E. coli* O157 from the soil by leaching or via surface run-off could result in the contamination of private water supplies.

Bacterial cells added in an innoculum (eg, in slurry or manure) may have different survival and movement characteristics to indigenous organisms already established within the soil matrix. Bacteria can move in soil either by active motility, by diffusion (Brownian motion) or in association with vectors. Four vectors contribute to the transport of bacteria through soil: namely, water, soil animals; growing plant roots; and fungal hyphae. Of primary importance is transport associated with percolating water. The presence of cracks, root or earthworm channels in soil can result in preferential flow of water through the soil profile, where percolating water by-passes the bulk of the soil matrix. Under these conditions, there will be limited exchange of bacteria in the percolating flow with water already held within the soil matrix.

The movement of bacteria in soil is dependent upon the availability of a continuum of water-filled pores, which is a function of soil structure (pore size distribution and arrangement) and matric potential. Within soil, there is a range of pores with neck diameters from less than one micron to several millimeters. Bacterial exchange occurs mainly in the larger pores (3 -> 30 µm); the water in pores smaller than 3 µm is generally considered inaccessible to bacteria (due to their size) and represents an immobile phase. The affinity of water to the soil matrix is termed the matric potential and is measured as the negative pressure (suction) required to remove water from the soil pores. For each soil, there is a relationship between the matric potential, the soil water content and the neck diameter of pores which are water-filled. This relationship can be determined experimentally from the moisture release characteristics of the soil.

Many factors affect the movement and survival of bacterial inoculum in soil including temperature, pH, carbon and nutrient status, soil moisture content, the presence of plant roots and soil animals, soil type and structure. The cell surface characteristics of the organism in question may also affect bacterial survival and movement capabilities. *E. coli* O157 may survive for longer periods in soil in comparison to other non-O157 isolates. Experimental information considering the survival characteristics of *E. coli* O157 is lacking in the literature, and further studies of this nature are required.

The soil environment represents an extremely hostile environment for bacteria where energy and nutrient reserves may be limiting, water stress is common and generally, temperatures are sub-optimum for gut-inhabiting organisms. Under conditions such as these, it is important to consider not only the culturable population of bacteria but also those that may no longer be culturable by methods routinely used for cell isolation e.g. through injury or development of a dormant, non-culturabale state. A number of *E. coli* strains including O157 and other pathogens (eg, *Vibrio vulnificus*, *Shigella dysenteriae*, *Campylobacter jejuni*) have been shown to be non-culturabale under certain conditions.

Development of a non-culturabale state by *E. coli* O157 in the soil environment requires elucidation, and then these findings need to be considered in the context of whether non-culturabale bacteria may act as a possible source of infection. Ideally, a range of methodologies should be used to assess whether non-culturabale populations exist for *E. coli* O157.

When added to soil, *E. coli* O157 populations represent a small proportion of the total soil microbial biomass making study of the organisms’ survival and distribution, problematic. In this study, reporter gene technology has been used to construct a *lux*-modified *E. coli* O157 strain. *Lux* genes encode expression of bioluminescence, and light emission in *lux*-modified cells has been shown to be directly proportional to cell metabolic activity (Meikle et al., 1992). Bioluminescence can be easily measured in soil using a luminometer or a sensitive light detecting camera (charged coupled device (CCD)) which provides a spatial element to detection. The advantages of using *lux*-modified bacteria include ease of detection, assessment of spatial distribution and specificity for the marked organisms as no other organism within the soil environment possesses the genes for bioluminescence (Prosser et al., 1996).

A simple microcosm system will be used to investigate the survival and movement of *E. coli* O157 in soil to allow a greater understanding of the mechanisms which control cell distribution in soil. Rainfall events will be simulated.
using a nebuliser system, and the leachate analysed for numbers and activity of lux-modified *E.coli* O157. A range of carrier materials (e.g. slurry, manure) can be used to add the *E.coli* O157 population. Results obtained from this project will complement field studies being carried out under another SOAEFD grant (Dr D. Fenlon, SAC, Craibstone, Aberdeen), and together the information will contribute to the development of a model to predict the fate of *E.coli* O157 in soil under a range of environmental conditions.

In conclusion, experimental studies are underway to provide information on the survival and movement of *E.coli* O157 populations in the environment. These studies will consider not only the culturable populations, but also assess whether non-culturable cells exist under environmental conditions. The information generated from this and related studies will be used to develop a model to predict the fate and movement of *E.coli* O157 applied to soil.

References


Jack McHardy (now Ritchie), Lauren Glover, Department of Molecular and Cell Biology, University of Aberdeen; K Killham, Y Beaton, Department of Plant and Soil Science, University of Aberdeen.
Survival of *E. coli* O157 in soil and water

DR Fenlon, A Vinten, D Lewis and ID Ogden

The principal problem in determining the rate of survival of *E. coli* O157 in soil and water following the application of animal excreta to soil is the sporadic incidence of the organism and the low numbers usually present. Additionally, these organisms are significantly outnumbered by other *E. coli* with similar growth characteristics, making the direct determination of survival rates under field conditions extremely difficult. However, the Scottish Agricultural College (SAC) does have field-scale lysimeters currently being used in unrelated slurry application experiments, and it was possible to add these *E. coli* studies to that programme. The aims of this study were to tackle the problem indirectly by: determining the fate of faecal coliforms in soil and water following the application of cattle excreta to land; establishing the relationship between the survival of faecal *E. coli* strains and *E. coli* O157; and in the long term develop a model to explain the fate of *E. coli* O157 in soil and water following application of cattle waste to land.

The first field trial was carried out on 9 March 1999 at Penicuik, Midlothian with the application of 19.6 m³/ha of dairy cattle slurry, 5% dry matter, to four drained plots on a clay loam soil, two in spring barley stubble and two in grass. The plots each had a nominal area of 600m² and were instrumented with tipping bucket flowmeters and water sampling devices. Drainage from half of each plot and surface runoff from the other half was sampled. Composite water samples were taken covering the two to four day period prior to each collection date. This gave complete coverage of the water quality during the trial period, when samples were collected at least twice weekly. Soils were sampled in the 0-2.5cm layer at days 1 and 3 after slurry application, and also at 0-2.5cm, 2.5-5cm and 5-20cm layers 12 days before slurry application and 7, 14, 21, 29, 35 and 49 days after slurry application. Grass was sampled at 7, 21, 35 and 49 days. The slurry contained 5.3 x 10⁵ *E. coli* g⁻¹ slurry, giving an application rate of about 10⁸ *E. coli* m⁻². Estimated *E. coli* O157 application was 660 cfu m⁻².

Soil recovery of *E. coli* was near 100% for the first three days and thereafter declined to less than 1% after 29 days. There was significant transport to deeper layers, but this never exceed 2% of applied numbers. Transport to drains was mainly associated with rainfall events between days 3 and 7 after application, leading to a cumulative loss of *E. coli* to drains of 7±4% of applied *E. coli*. There was no significant difference in losses from the arable and grassland plots. Moore swabs did not pick up any *E. coli* O157 in the drainage water and soil samples were only positive for the first 7 days after slurry application. Grass samples showed 600 - 8800 cfu *E. coli* g⁻¹ after seven days and these day 7 samples also contained *E. coli* O157.

There were substantial numbers of *E coli* detected in drains and surface runoff in a storm event occurring between 10 and seven days before sampling. The largest loss was in surface runoff from one of the grass plots, which amounted to 1.6% of the *E. coli* subsequently applied in the slurry.

In laboratory studies the survival of *E. coli* O157 in slurry was compared to the existing population of *E. coli* by adding approximately equal numbers of an *E. coli* O157 Phage Type (PT) 2 strain previously isolated from cattle slurry. Over a 100 day period *E. coli* numbers dropped by 2 (6°C) to 2.5 log cycles (15°C), *E.coli* O157 numbers at these temperatures fell by 3 to 4 log cycles respectively. However a pronounced tailing effect was noticed particularly with *E. coli* O157 once numbers fell below 10 cfu g⁻¹. Survival of *E. coli* and *E. coli* O157 PT2 and PT21/28 was compared in stream water, *E. coli* O157 was near 100% for the first three days and thereafter declined to less than 1% after 29 days.

Conclusions

- The risk to the human population from *E. coli* O157 from faecal sources can be minimised by the testing of water for faecal coliforms, as the latter have similar or slightly longer survival times than *E. coli* O157.
- Application of slurry following appropriate guidelines should result in most *E. coli* O157 being retained in the soil matrix. *E. coli* O157 has similar survival rates in soil and slurry to other faecal strains of *E. coli*.
- In this study we observed losses of about 7% of applied bacteria to drain water over the first two weeks.
following application. This was a dry period and losses could be higher with heavy rain. However, this will also result in dilution and die off is more rapid in water than soil.

The recommended three-week ban on grazing or harvesting grass following slurry application allows most applied \textit{E.coli} to die off or be washed off the foliage. Numbers of \textit{E.coli} in the top one inch of soil still remained higher than the background count after this time. An increase in the ban to four weeks might be justified if this is finding is confirmed.

The risk from grazing animals, with sustained contamination of the grass and soil with fresh faeces, which can have higher \textit{E.coli} O157 counts, has yet to be assessed.

\textbf{Acknowledgement}

This study is funded by the Scottish Executive Rural Affairs Department (SERAD).

\textbf{DR Fenlon}, SAC Veterinary Science Division, Aberdeen; \textbf{AVinten}, SAC, Edinburgh; \textbf{D Lewis, ID Ogden}, Applied Food Microbiology Group, Department of Medical Microbiology, University of Aberdeen.
Escherichia coli in farm animals

CS Stewart

Ruminants and other farm animals are considered to act as reservoirs for enterohaemorrhagic Escherichia coli. Reducing the carriage and faecal shedding of this bacterium by ruminants would be expected to decrease the incidence of contamination of meat, vegetables, fruit, milk and water entering the human food chain. There is also evidence for direct transmission of disease to farm workers and their families. This may occur through direct contact with infected animals, or from contact with contaminated carriers ranging from farm pets to farm machinery.

Changing the diet is the most obvious way in which the proliferation of certain ruminant gut bacteria might be controlled. However, there is as yet no agreement on whether grain-based or fibre-based diets pose the greater risk of sustaining high shedding of E. coli O157. Furthermore, it is not clear whether producers could, at acceptable costs, radically alter the composition of production diets. It is possible that some feed additives or supplements such as growth promoters might tend to encourage proliferation of E. coli. Identifying specific examples and reducing their use could be helpful. Modern production and slaughtering systems may specifically increase the opportunities for contamination of meat in the slaughterhouse, and changes of practice may lead to greater control of the potential for infection. Food is normally withheld from animals immediately before slaughter. Under these conditions, the numbers of E. coli in the gut may rise, as a consequence of declining activity of the anaerobic rumen bacteria. Increased shedding of E. coli, at a time when animals may be under transport stress and held in close confinement, poses a risk of animal-to-animal transmission, amplifying the potential for contamination of the environment and the food chain.

Controlling the proliferation of E. coli in the gut during the period immediately before slaughter is thus seen as an important research target.

Work at the Rowett Institute seeks to evaluate the key microbial, dietary and physiological factors which may influence the survival of E. coli in the ruminant gut and the shedding of this bacterium in ruminant faeces. Understanding the ecology of E. coli in the ruminant gut is a central aim. In the rumen, which provides up to 70% of the gut volume of cattle and sheep, the viable numbers of E. coli represent less than 0.001% of the total bacterial population of around 10^{11} bacteria per ml. Earlier experiments with K12 derivatives suggested that the concentration of volatile fatty acids (VFA) in the rumen and colon limit the proliferation of E. coli in the bulk phase of the digesta. However, this is probably an oversimplification. We have shown that some commensal strains of E. coli isolated from the rumen, and the O157:H7 serotype reference strain NTCC 12900, can grow much faster than the typical rumen liquid phase dilution rate in batch cultures in the presence of 100 mM VFA. Other experiments\(^1\), suggest that the population size of E. coli in the liquid phase of the gut digesta is likely to be governed mainly by competition for nutrients, unless the VFA concentration reaches around 150 mM. Some strains of E. coli, including strain 12900, may attach to rumen and colonic wall tissue, potentially implicating the wall-attached biofilm as a habitat for this bacterium. The localisation of E. coli in the different phases of the ruminant digesta, a key factor affecting the rate of shedding of this bacterium in the faeces, is being studied using antibiotic resistant mutants and cells expressing gfp. This information will be valuable for modelling shedding, and will complement data on the occurrence of E. coli in different gut compartments of naturally-infected cattle which is being obtained by colleagues at ADAS\(^8\).

Rumen bacteria may affect the growth of E. coli by producing specific inhibitors, competing for attachment sites, or metabolising components of the diet, releasing metabolites specifically inhibitory to E. coli. Evidence has been obtained that all of these mechanisms may be operating in the gut. Screening rumen isolates for the ability to inhibit growth of E. coli revealed the presence of a range of bacteria inhibitory to the growth of test strains of E. coli O157. So, far, the best characterised among these bacteria are strains of Pseudomonas aeruginosa, which produce compounds inhibitory to the growth of E. coli and which also adhered strongly to rumen epithelial cells grown in vitro\(^4\).

A novel mechanism of inhibition of the growth of E. coli by anaerobes has recently been discovered. The hydrolysis of the natural plant coumarin glycoside esculin by anaerobic gut bacteria results in the release of the aglycone esculetin. Esculetin has a selective inhibitory effect on the growth of E. coli, with little effect on most anaerobes tested so far\(^3\). Understanding the mechanism of this effect may provide new strategies for the control of the proliferation of E. coli in the gut\(^1\).

Future research priorities include the following:

- using data on localisation to more accurately model shedding;
- identify whether currently-used dietary additives or supplements enhance the survival of E. coli.
O157 in the gut, with a view to reducing their use;

- establish the mode of action of inhibitors and antagonistic bacteria, and investigate their use to control proliferation and survival of E.coli;
- identify suitable harmless strains of E.coli for use as models, reducing the need to handle pathogens;
- investigate the potential for new approaches to on-farm disinfection arising from studies on the effects of organic acids.

Rowat Research Institute receives financial support from the Scottish Executive Rural Affairs Department (SERAD). The future investigation will be carried out with the aid of a grant from the Ministry of Agriculture Fisheries and Food (MAFF).

References
6. R. Laven, personal communication

C Stewart, Scottish Centre for Infection and Environmental Health, Glasgow.
SAC Cattle Studies on *Escherichia coli* O157

**BA Synge, HE Tement, G Foster, I McKendrick and GJ Gunn**

**Introduction**

Two major studies are nearing completion. The first, funded by the Ministry of Agriculture, Fisheries and Food (MAFF), is to determine factors influencing the shedding of *E. coli* O157 in farm animals. The second, funded by Scottish Executive Rural Affairs Department (SERAD), is to determine the prevalence of the organism in Scottish Beef Cattle. The progress with these studies is described and brief mention is made of the investigation carried out for the recent incident at Applecross in the Highland Region.

**Factors influencing the shedding of *E. coli* O157**

This three year study is due to finish in March 2000. Thirty-two herds of beef suckler cows are being visited. Sixteen of the herds had previously had positive animals on the farm, while 16 others were of unknown status. Bovine faeces are collected at approximately monthly intervals, but visits coincide with management procedures such as calving, housing, turnout, weaning or feed changes. Records are kept of events on the farm by the completion of questionnaire forms at each visit. Since there are still 20 farms being sampled it is too early to try to relate changes in shedding to management practices. It has been noted, however, that 24 out of the 32 herds have shed *E. coli* O157 at some stage in the sampling. This cannot be taken as the herd level prevalence for suckler cows, since herds were selected on the basis that half of them had previously been positive.

**Determination of the prevalence of *E. coli* in Scottish Beef Cattle**

The major part of this study is to estimate the herd level prevalence of verocytotoxin-producing *E. coli* O157 for fattening cattle in Scotland using the immunomagnetic separation (IMS) technique on 1 gram faeces samples. It is very important to define the methods to enable any comparisons to be made with other studies. In an earlier study (1992-93) by the Scottish Agricultural College (SAC), using direct culture methods, 0.25% of 5237 cattle samples were positive for *E. coli* O157. In a similar study in England and Wales (1994-5) the Veterinary Laboratories Agency (VLA) found a prevalence of 0.83% using IMS. Chapman (1993) working at the Sheffield slaughterhouse found 4% animals shedding by direct culture and later by IMS found 15.7% positive (Chapman 1997). In both cases, IMS gave four times the prevalence compared to direct culture, but the comparisons are not valid as the studies were in different populations at different times.

A pilot study was completed that demonstrated the exclusion of antibiotics from the primary enrichment broth significantly increased isolation rate. This more sensitive method was therefore used for the study. While collecting samples from 900 farms throughout Scotland the opportunity is being taken to collect as much epidemiological data as possible. The questionnaire used has been carefully validated using two operators. As a sub-objective, the animal level prevalence within positive herds will also be determined.

A weighted cross-sectioned survey of the slaughter cohorts from over 900 herds (dairy, beef and specialist finishing units) is nearing completion. Weighting was for management type and region. The results will be available in summer 2000 and it will be possible to analyse the figures on a regional, seasonal and herd management basis.

**The Applecross incident**

Details of this outbreak will be published elsewhere. The outbreak affected people who had been on a campsite in the Western Highlands. The water supply for the campsite was found to be positive for *E. coli* O157. Although at first this was thought to be spring water it turned out to be surface water which had run underground for some distance. Sheep faeces collected from the hillside, within the water catchment area, were positive for *E. coli* O157. The phage type and pulse field type of this isolate was indistinguishable from that isolated from the water and the campers, while separate types affected those who had stayed at a neighbouring cottage.

**Acknowledgements**

The financial support of the Ministry of Agriculture Fisheries and Food (MAFF) and Scottish Executive Rural Affairs Department (SERAD) is gratefully acknowledged.

Wellcome Trust Project

MEJ Woolhouse

The Wellcome Trust’s International Partnership Research Awards in Veterinary Epidemiology (IPRAVE) scheme is a special initiative designed to strengthen research capacity relating to public health problems associated with zoonotic or food-borne pathogens. One of the two awards made last year was for a project entitled ‘Epidemiology and evolution of Enterobacteriaceae infections in humans and domestic animals’. This project is a collaborative venture involving institutions in Scotland, elsewhere in Europe and North America. The project has a budget of over £3M over five years and involves more than 30 researchers. Work began in July 1999.

The project will focus on *E. coli* O157 and other VTEC and on *S. typhimurium* DT104 and other salmonellae. There are three main aims:

- to understand the epidemiological relationships between enterobacteria in different hosts and host populations looking at spatiotemporal distributions, molecular epidemiologies, transmission routes and risk factors, and the question of persistence of enterobacteria populations at different spatial scales;
- to study the distribution and movement of antibiotic resistance and virulence factors between different hosts and host populations, looking at population genetics in the field and using *in vitro* and *in vivo* experimentation;
- to explore the likely impact of changes in the management of enterobacteria infections – by developing statistical and mathematical models of the dynamics of these infections.

There are seven component subprojects:

1. Transmission dynamics in the field (M Woolhouse, C Low, G Gunn and B Synge): epidemiological studies on Scottish beef cattle farms.
2. Pre and post harvest risk identification and quantification of foodborne transmission (S Reid, W Reilly and S McEwen): forward and backward tracing from farm to human outbreaks.
3. Transfer of antibiotic resistance and virulence genes between host populations (S Amyes): molecular genetics studies of field material.
4. Novel molecular approaches to study enterohaemorrhagic *E. coli* (EHEC) (G Dougan and G Frankel): molecular biology of *E.coli* O157 and other VTEC.
7. Overview and central activities (M Woolhouse): maintenance of central library of biological material and central database.

Altogether, the Partnership involves the following institutions: University of Edinburgh Veterinary School; University of Edinburgh Medical School; University of Glasgow Veterinary School; Imperial College, London; Emory University, USA; Scottish Agricultural College; SCIEH; University of Guelph; Scottish Reference Laboratories; Biomathematics and Statistics Scotland; PHLS; University of Birmingham; University of Maryland, USA; IHAID, Germany; CDC, USA; and WHO, Switzerland.

Further details will be shortly available on the project’s web site.

MEJ Woolhouse, Centre for Veterinary Tropical Medicine, Royal (Dick) School of Veterinary Studies, University of Edinburgh.
Poster presentation:

A comparison of Verocytotoxin-producing Escherichia coli O157 phage types isolated in England and Wales with those from 13 other European countries: January 1997 to June 1999

T Cheasty, F Allerberger, L Beutin, A Caprioli, A Heuvelink, H Karch, S Lofdahl, D Pierard, F Scheutz, A Siitonen and H Smith

Verocytotoxin-producing strains of Escherichia coli O157 (VTEC) are an important cause of both bloody, non-bloody diarrhoea and haemolytic uraemic syndrome (HUS). In England and Wales the number of isolates of E.coli O157 VTEC having risen from two in 1983 to 890 in 1998. The need to monitor the increasing spread of infection with this organism both in outbreak situations and amongst sporadic cases has been facilitated with the use of phage typing. There have been gradual changes in the predominant phage types associated with human infections in England and Wales observed during this period with PT2 remaining dominant and PT49 showing a marked decline. The information presented shows the phage typing results for the two and a half year period from January 1997 to June 1998 as part of the continued surveillance of O157 VTEC infections in Europe by the Laboratory of Enteric Pathogens (LEP), Central Public Health Laboratory.

Cultures received from participating countries were plated on to blood agar for purity and a single colony selected for biochemical identification and serotyping and confirmed as VTEC using DNA probes specific for VT genes. For phage typing, strains were grown in Difco nutrient broth, incubated for 1.5 hours at 37°C in an environmental shaking incubator and phage typed using the E. coli O157 phage typing scheme of Ahmed et al. extended by Khakhria et al. This scheme consists of sixteen bacteriophages and now recognises over 80 phage types.

During the period January 1997 to June 1999, the LEP received 3804 isolates of Verocytotoxin-producing E.coli O157 from laboratories in England and Wales and 1016 from 13 European countries (Austria, Belgium, Czech Republic, Denmark, Ireland, Finland, Germany, Greece, Italy, Netherlands, Northern Ireland, Spain and Sweden) for phage typing. They comprised 2978 isolates from humans, 1443 from animals (cattle, sheep, goats, pigs, deer, horses and a kitten), 214 from human foods, seven from dog food and 75 environmental isolates. All 4717 strains gave patterns of lysis when phage typed. Thirty-six phage types (PT) were identified (the international scheme recognises >80 phage types). The predominant PTs among the human isolates were 2, 4, 8, 14, 21/28 and 32. These phage types were also found in the animal and food isolates. There was a distinct association between some of the predominant phage types of the human isolates with different countries eg PT2 in England & Wales, and Finland, PT4 in Denmark and PT32 in Ireland. In Belgium and Germany, two PTs, 2 and 8, were of equal prominence and in Sweden it was PTs 4 and 8.

Phage typing is a valuable tool enabling the rapid identification of outbreaks and epidemiological trends. As part of the European VTEC study, 4717 strains of O157 VTEC from thirteen European countries were phage typed. The majority of the strains were human faecal isolates (48%) and 29 phage types were identified among them. The most common phage types amongst these strains were PTs 2, 4, 8, 14, 21/28 and 32. Amongst the 1443 animal isolates 26 phage types were observed, the majority similar to those isolated from man. Phage types 2, 4, 8, 14, and 32 were found amongst isolates from human, animals or both in most of the participating countries of this study. The strains designated as RDNC will, after further study, be assigned as new phage types.

Predominant Phage Types and Country: Human isolates

<table>
<thead>
<tr>
<th>PT</th>
<th>England &amp; Wales</th>
<th>Belgium</th>
<th>Denmark</th>
<th>Finland</th>
<th>Germany</th>
<th>Ireland</th>
<th>N. Ireland</th>
<th>Sweden</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>32%</td>
<td>26%</td>
<td>19%</td>
<td>75%</td>
<td>34%</td>
<td>7%</td>
<td>13%</td>
<td>35%</td>
</tr>
<tr>
<td>4</td>
<td>7%</td>
<td>14%</td>
<td>31%</td>
<td>7%</td>
<td>28%</td>
<td>26%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>17%</td>
<td>26%</td>
<td>8%</td>
<td>28%</td>
<td>26%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>19%</td>
<td></td>
<td>12%</td>
<td>6%</td>
<td>22%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21/28</td>
<td>19%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>81%</td>
<td>45%</td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>8%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>81%</td>
<td>45%</td>
<td></td>
</tr>
<tr>
<td>49</td>
<td>12%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4%</td>
<td>14%</td>
<td></td>
</tr>
</tbody>
</table>

References

7. R.Khakhria, personal communication.

T Cheasty, H Smith, Laboratory of Enteric Pathogens, Public Health Laboratory Service (PHLS), Colindale, London; F Allerberger, Institute for Hygiene, Innsbruck, Austria; L Beutin, Robert Koch Institute, Berlin; Germany; A Caprioli, Istituto Superiore di Sanita, Rome, Italy; A Heuvelink, University Hospital Nijmegen, The Netherlands; H Karch, Institut für Hygiene u. Mikrobiologie, der University, Wurzburg, Wurzburg, Germany; S Lofdahl, Swedish Institute for Infectious Disease Control, Solna, Sweden; D Pierard, Akademisch Ziekenhuis Vrije Universiteit Brussel, Brussels, Belgium; F Scheutz, International Escherichia and Klebsiella Center, Statens Serum Institut, Copenhagen, Denmark; A Siitonen, Laboratory of Enteric Pathogens, National Public Health Institute, Helsinki, Finland.
Mucosal and systemic immune responses to the lipopolysaccharide of

E. coli O157

CG Currie, K McCallum and IR Poxton

E. coli O157 and other verotoxin-producing E. coli (VTEC) are now well recognised as being major causes of morbidity and mortality in the young and the elderly. Infections caused by these strains, including haemorrhagic colitis and haemolytic uraemic syndrome (HUS) appear to be more common in Scotland than the rest of the UK. Because of increased incidence at the extremes of age there is a suggestion that immunity may influence susceptibility to disease, and it is likely that the mucosal defences may be more important than systemic immunity. These strains of E. coli express several antigenic virulence factors including lipopolysaccharide (LPS). LPS is recognised as being involved in interactions with host cellular functions including the possibility of a synergistic interaction on the uptake and action of the verotoxin, by inducing up regulation of Gb3 (the receptor for verotoxin). It is also a likely candidate for inducing protective immunity.

The LPS of E. coli contains two major antigenic components, the serotype specific O-polysaccharide and the core oligosaccharide. Five different LPS cores have been identified in E. coli (R1-R4 and K12) and, although similar to each other, can be distinguished serologically because of the arrangement of the terminal sequence of hexoses and hexosamines. All VTECs appear to have R3 LPS cores.

In this study we investigated the humoral immune responses to the O157 LPS and the R3 core oligosaccharide in the general population and patients convalescing normally from E. coli O157 infection. We have characterised both the systemic (IgG and IgM) and the intestinal (IgA) antibody responses to these two antigens. Mucosal antibodies were measured using whole gut lavage fluid (WGLF) which is a whole gut perfusion that contains mainly IgA in healthy individuals.

Serum and WGLF was obtained from convalescent patients (n = 9) ranging from eight weeks to 113 weeks post infection; healthy volunteers (n = 20), age range 18-75 years; and a group of GI patients (n = 53) who had WGLF samples taken for routine clinical investigations but were subsequently shown to be immunologically normal. Serum alone was obtained from an additional 26 healthy volunteers and 13 convalescent patients. We also examined serum from a group of slaughterhouse workers (n = 10) and, in order to establish the natural levels of serum antibodies in the general population in Edinburgh, we screened a large number of serum samples from healthy blood donors (n = 647). Measurement of antibodies was determined by ELISA on microplates coated with polymyxin complexes of LPS extracted from E. coli O157 or an R3 core type rough mutant strain of E. coli.

A lack of co-operation by certain Health Authorities during this study prevented us obtaining acute sera and as many early samples from convalescent patients as expected. This has seriously limited the data available. However it is apparent that, in health, antibodies to both O157 LPS and R3 core are detectable in serum (IgG and IgM) and WGLF (IgA), and that levels can vary greatly between individuals. Notably, there are some individuals in the general population with very high levels of IgG to R3 core (three- to eight-fold greater than the mean) and O157 LPS (three- to four-fold greater than the mean). The plasma from these hyperimmune individuals might be collected and used for passive immunisation.

Despite the low number of samples obtained, the study found that convalescent patients have significantly higher levels of mucosal IgA to both O157 LPS and R3 core compared with healthy volunteers, indicating that within our patient group (all of whom recovered normally from E. coli O157 infection) mucosal antibodies to both antigens were produced. This may have had a protective effect and prevented serious complications occurring. The main humoral response observed in VTEC infections is an IgM response; yet, these patients did not have significantly higher levels of serum IgM or IgG to either antigen than the healthy volunteers. This may be accounted for by the fact that that the majority of our convalescent group were more than 14 weeks post infection and their IgG, and most certainly IgM, will have returned to normal levels.

It should be stressed that purified O157 LPS contains both the O157 O-polysaccharide and the R3 core oligosaccharide. In assessing how much of the O157 LPS response was to the O157 polysaccharide and how much was to the R3 core, we found that the serum IgG response to O157 LPS contained antibodies specific for both antigens, but that in the convalescent patient group, the majority of the IgG response was to the O157 polysaccharide.
The relationship between cattle cleanliness and carcase contamination

L Humpheson and J Bruce

Introduction

Dirty livestock have been identified as a source of carcass contamination. In order to combat this source of contamination, the Meat Hygiene Service (MHS) has introduced a five-category scoring system whereby excessively dirty cattle are excluded from the food chain.

The main concern is that organisms such as *Salmonella*, *Escherichia coli* and other faecal pathogens gain access to the carcase either from the dirty hide or other sources during slaughter. Once on the carcase, these pathogens are likely to pass into the food chain and act as potential sources of food poisoning bacteria.

Objective

To establish if a relationship exists between visual contamination of cattle and faecal contamination of subsequent carcases.

Experimental Approach

In this study, *E.coli* levels on carcases were used as an indicator of faecal contamination. These contamination levels were correlated with visual hide cleanliness scoring of the animals before slaughter.

Hide Cleanliness Assessments

Permission to access three Scottish abattoirs (designated A, B and C) over a period of 18 months was obtained. A scoring system was developed to assess the total hide contamination of cattle at the time of slaughter. Selected animals were rated one to five (with five being the heaviest contamination) for the following regions:

- Fore feet
- Fore legs
- Neck
- Flank (right)
- Tail
- Hind belly
- Hind feet
- Hind legs
- Flank (left)
- Rump
- Fore belly
- Brisket

Animals which had the very minimum visible contamination of the hide would score 12, whereas those receiving a maximum contamination rating for each region would score 60.

Bacteriological Assessment

Those animals scored and selected for bacteriological analysis were followed along the slaughter line and one half of the now split carcases were swabbed after MHS inspection and health-marking but prior to washing. Swabbing employed a 100 mm high density foam roller (manufactured by Mosley-Stone, Leeds UK), soaked in thio-sulphate Ringers buffer and autoclaved at 121°C for 15 minutes, mounted on a long-reach decorator’s paint roller. Bacteriological contamination on the selected carcase-sides was sampled by rolling the swab over the subcutaneous surfaces.

On return to the laboratory, each swab was placed in a stomacher bag and combined with 50 ml of maximum recovery diluent (MRD) and hand-massaged for one minute. These neat suspensions of the swab contents were then diluted tenfold in nine ml MRD volumes for inoculation of three-tube MPN in triplicate. Growth and gas production in lauryl sulphate broth and Eijkman confirmation were used to determine the presence and level of *E.coli*.

Results

Monitoring hide contamination during abattoir visits enabled the comparison of SAC total hide scores with the guidelines issued by the MHS. It was concluded by observation that animals scoring up to 19 corresponded to MHS contamination category one. Cattle scoring between 20 and 29 corresponded to MHS contamination category two, and those scoring between 30 and 39 corresponded to MHS contamination category three. Animals amassing scores of 40 and over were assessed to fall into MHS contamination category four or five. No animals selected in the study fell into category five.

It was found that in abattoirs A and B, 90 and 92% respectively, of animals sampled fell into MHS categories one and two. Only a small portion processed fitted the description of MHS category three animals. The levels of *E. coli*...
recovered from these carcases appeared not to show any particular trend, regardless of initial hide contamination, thus the carcases from clean animals often returned *E. coli* levels as high as those from animals sampled in the MHS category three grouping.

At abattoir C (over thirty months (OTM) cull abattoir), *E. coli* counts were generally higher, though again there were some very low levels picked up from some carcases. Cattle processed at this abattoir also tended to carry more contamination on their hides. It is suspected that the greater proportion of category three and over animals is not responsible for the higher *E. coli* count of subsequent carcases, but instead, the less-stringent processing controls applied at the plant, for these cull animals not destined for the food chain.

When data for each abattoir were analysed by averaging the *E. coli* counts of each animal for MHS categories one to four, it became apparent that abattoir A had significantly lower levels of *E. coli* contamination than abattoirs B and C (P > 0.005). No significance was observed between abattoirs B and C (P < 0.05). Although in abattoirs A and B there was a trend for *E. coli* levels to correlate with the contamination scores of the cattle, both abattoirs exhibited low and high levels of contamination in animals scored in category 1, indicating that abattoir practices are probably having a significant influence on carcass contamination levels. Comparing *E. coli* levels between abattoirs revealed that abattoir A had not only the lowest mean *E. coli* level of all cattle sampled but also had the lowest mean *E. coli* counts category by category. Although these differences may be attributable to differing levels of hygienic practice between abattoirs, other factors such as time of year visits were carried out and the origins of the cattle slaughtered at each abattoir may also have influenced the carcass *E. coli* counts. It should also be noted that at abattoirs A and B, the relative numbers of cattle falling into MHS category three is small compared to categories one and two, making direct comparison with mean *E. coli* counts of category three animals with those of category one and two less valid.

**Conclusions**

- The MHS scheme is controlling the number of dirty animals going to slaughter.
- Category 1 and 2 cattle tend to give carcasses with lower *E. coli* levels than category 3 animals.
- The MHS cut off point at category 3 is justified.
- Given a reasonably uniform standard of cleanliness of cattle, abattoir practices are likely to be the major influence on the *E. coli* contamination levels on the final carcass.
- Using the swab technique described a level of 100 *E. coli* per swab is achievable and could be adopted as a target value.
- Levels between 100 and 1000 per swab could be considered as acceptable given current conditions in abattoirs.

**References**


**Acknowledgements**

The authors wish to thank the management and staff of the abattoirs involved in the study for their assistance. This project was funded the Scottish Executive, SAC CR644009.

L Humpheson, J Bruce, SAC, Auchincruive.
The mucosal immune system consists of specialised local inductive sites and widespread effector sites, both of which are separated from mucosal surface antigens by epithelial barriers. Isolated and aggregated organised lymphoid follicles (mucosal-associated lymphoid tissue, or MALT) are distributed throughout the follicle-associated epithelium (FAE) providing areas where the collaboration of epithelial cells with antigen-presenting and lymphoid cells is highly developed. The FAE overlying lymphoid follicles contains a distinct set of specialised intra-epithelial cells called M (membranous) cells that are believed to play a key role in mucosal immunity. M cells continuously sample particulate foreign material and deliver it via an efficient vesicular system to the intra-epithelial lymphoid cells that process antigens to generate a protective immune response. Unlike adjacent enterocytes, M cells have few or no lysosomes, therefore transported antigens avoid degradation. Morphologically, M cells are described as lacking an overlying mucus layer and may be identified by their poorly organised brush borders and sparse microvilli. They are also characteristically invaginated basolaterally to form an intracellular lymphocyte-containing pocket. Markers such as vimentin and cytokeratin can be used to identify M cells in various laboratory animal models.

Vaccines that preferentially stimulate the mucosal immune response to provide an effective barrier against pathogens are highly desirable due to the fact that mucosal surfaces are the most common site of pathogen entry, and that over 90% of all infections are acquired by mucosal routes. The goal of this study is to design a successful novel mucosal vaccine by targeting M cells and MALT. There are many advantages to targeting M cells and MALT over systemic vaccine delivery. Firstly, mucosally derived antigens are highly efficient because of their ability to stimulate both mucosal and systemic responses, thereby inducing local protection at mucosal surfaces, creating a barrier, while simultaneously targeting the pathogen with the systemic response. Secondly, the existence of a common mucosal immune system has now been established, meaning that specific antigen-activated lymphocytes from one region of MALT can disseminate to various other mucosal and glandular tissues, producing a disseminated mucosal immune response. Therefore, antigen delivery to one region of the mucosal tract of a young animal will induce immunity throughout the mucosal surface. Finally, mucosal immune responses do not suffer from age related immune dysfunction.

The proposed delivery for the vaccine is the intra-nasal/respiratory route. The NALT in the nasal mucosa offers many advantages as a route for vaccine delivery over the oral route. It is a readily accessible, highly vascularised, leaky epithelium of considerable surface area where antigenic degradation is reduced due to the less aggressive physiological conditions than those present in the GI tract, ie, lower enzymatic activity and less extremes of pH. The total antigen load is less in the nasal cavity than in the GI tract and the antigen has immediate contact with NALT, allowing greater sensitivity and increasing antigen uptake. Previous studies have shown that intra-nasal application of vaccines is more efficient and more effective than intra-gastric immunisation at generating earlier and stronger generalised mucosal immune responses, and NALT also appears capable of retaining long-term memory.

The cellular and molecular features that promote adherence and transport of antigens and microorganisms across the epithelial barrier are crucial in the design of mucosal vaccines. Distinct carbohydrate receptors on the surface of M cells may be involved in the selective binding of certain micro-organisms. Studies in rodents have shown that these receptors can be targeted by lectins, for example, *Ulex europaeus* 1 specifically targets mouse Peyer’s patch M cells when they are fixed and *in vivo*. However, lectin binding varies from species to species and also regionally in the same species. For example, it has been shown in the mouse that certain lectins bind to M cells of the Peyer’s patches but not the caecum and vice versa. Studies on M cell specific lectin-staining on human non-inflamed appendices suggest that in humans there are even strong inter-individual variations in lectin binding sites on M cells. As yet there have been no published data regarding lectin targeting in the sheep, but preliminary studies performed at Moredun Research Institute (MRI) have shown that FAE within the nasal region and lower respiratory tract are selectively stained with FITC-succinylated wheat germ agglutinin.

It is envisioned that the antigen, together with the lectin that best targets M cells, will be incorporated into a microsphere delivery system. The use of microparticles for vaccines offers improvements in the protection and delivery of peptide antigens as well as presentation to the local immune system, facilitating the induction of both a humoral and cytotoxic T cell response. The uptake of microparticles is influenced by various physicochemical properties including their size (50-300nm are capable of uptake and translocation), surface charge, hydrophobicity and the presence of attached ligands such as lectins. Most studies using microparticles as antigen delivery systems for mucosal vaccines have relied upon the use of the recently developed synthetic poly-D,L-lactide-co-glycolide (PLG) nanoparticles which are
biocompatible, biodegradable and easily manufactured in the laboratory\(^1\). These PLG microspheres will be used in the vaccine. The degradable microsphere containing the M-cell specific lectin and antigen will therefore target the area where mucosal immune responses are initiated, decreasing the amount of antigen needed and increasing the immune response, creating an effective barrier to the antigen when re-encountered.

**Proposed Line of Study**

The first objective is to determine the location of ovine nasal-associated lymphoid tissue, and also BALT or GALT in Peyer’s patches if necessary. The location will be confirmed by immuno-histochemical staining for T and B cells, followed by an attempt to identify and isolate M cells and determine the nature of their receptors. If attempts at M cell isolation are successful, a method to culture primary cell lines will be adapted from existing methods to culture epithelial cells. There is currently no method of culturing M cells, partly due to the limited understanding of factors mediating their development *in vivo*. One possibility is that the proximity to lymphocytes may be a pre-requisite for the expression of the M cell phenotype *in vitro*. This theory is supported by a study in which a human intestinal cell line was co-cultured with lymphocytes\(^5\). The epithelial cells gained M cell-like characteristics, including reorganisation of the epithelial brush border and associated proteins; and enhanced transport of inert particles and the non-invasive bacterium *Vibrio cholerae*. If primary cell lines are successfully set up, they will then be used to define the functional activity of M cells and to create a model system *in vitro*. Further studies with lectins shall be undertaken to elucidate which lectin specifically targets M cells in NALT. The lectin shown to target M cells most efficiently will then be incorporated into a microsphere, and optimal binding of these microspheres to M cells will be assessed with regard to size of particle, concentration of lectin, etc, in both *in vitro* and *in situ* studies. Finally two experimental proteins will be purified and utilised as antigens to analyse the response to the vaccine *in vivo*. These proteins are listeriolysin (LLO) derived from *Listeria monocytogenes* and SAG-1, a major surface protein of *Toxoplasma gondii*. Both organisms are important food-borne pathogens capable of infecting and causing serious illness in both sheep and humans. The protein will be incorporated into the microsphere with the lectin, and the final vaccine will be administered to the animal via an intra-nasal spray. The immune response to the vaccine will then be monitored and the methodology fully evaluated, giving a measure of the efficacy of this novel vaccination strategy and providing further information on the mucosal immune system.

**References**


JF Huntley, D Buxton, W Donachie, C Morrell, AC Stanley, Moredun Research Institute.
The Veterinary laboratories Agency (VLA) is undertaking a wide-ranging surveillance and research programme for the investigation of E. coli O157 in cattle and other animals. The majority of this work focuses on the strains pathogenic to people (i.e. verocytotoxigenic).

**Areas of Surveillance and Research:**

**Survey for foodborne pathogens excreted by cattle and sheep presented to abattoirs in Britain for slaughter for human consumption**

- **Project leader:** Jane Gibbens
- **Collaborators:** Meat Hygiene Service (MHS)
- **Funding:** MAFF-funded project (1998-2000)
- **Anonymous abattoir survey of animals slaughtered for human consumption**
- **One year sample collection period**
- **Results available mid-2000**
- 117 of the 393 eligible abattoirs which kill cattle and/or sheep
- Approximately 4000 samples of rectal contents from each species
- All samples are examined for VTEC O157
- 20% of samples examined for Salmonella, Campylobacter, commensal E. coli, enterocolitica and E. faecium.
- Antibiotic sensitivity profiles determined for all VTEC O157, Salmonella, E. coli and enterocolitica isolates.
- Questionnaire survey of all 393 abattoirs to assess participation bias established that the survey findings will be generalisable to larger abattoirs which draw animals from regional or national locations

**Surveillance of animal premises associated with human outbreaks of VTEC O157**

- **Project leader:** Jane Gibbens
- **Funding:** MAFF-funded surveillance programme
- VLA vets investigate animal premises epidemiologically associated with human outbreaks of VTEC O157 in England and Wales
- Implicated animal groups examined to establish if excretion is occurring on day of visit; adequate samples to detect 2% prevalence of excretion with 95% certainty
- Isolates typed to determine if distinguishable from human isolates
- General hygiene advice given
- Results collated annually

**Epidemiology of VTEC O157 in cattle**

- **Project leader:** Giles Paiba
- **Funding:** MAFF-funded project (1998 - 2002)
- To investigate the epidemiological aspects of VTEC O157 excretion by cattle
- **Prevalence study:**
  - To determine the prevalence of infected cattle and cattle herds in England and Wales
  - 90 randomly selected cattle farms (30 each of dairy, beef fattening and beef suckler herds)
  - Rectal faeces collected from randomly selected animals to detect with 95% certainty a prevalence of excretion of 2% ± 2%
  - Samples tested for VTEC O157
- **Longitudinal study:**
  - Farm and animal specific data collected using questionnaire for epidemiological analysis
  - Results available in spring 2000
  - To investigate the risk factors for excretion
Complete mid-2001

Intervention study:
To design and measure the effectiveness of on-farm management changes on the ability to reduce excretion of VTEC O157 by cattle
Complete by mid-2002

Plant antibody delivery of passive immunisation against *E.coli* O157:H7: a novel means of control in the animal

Project leader: Prof MJ Woodward
Collaborators: Mr J.B. Kilpatrick (ADAS Rosemaunde and Leicester University)
Funding: MAFF funded (1999 - 2002)
VLA is supplying candidate antigens for passive immunisation
Antigens include recombinant *eaeA*, *espA*, outer membrane and whole cell preparations
Cloning of antigen-specific antibody genes to produce transgenic plants
Animal models developed at VLA will be used to assess the efficacy of antibody delivery as a method for immunisation

A proteomic approach to identify virulence determinants of EHEC O157

Project leader: Dr C.D. O’Connor (University of Southampton)
Funding: MAFF funded (1999-2002)
Bovine ileal loop model and proteomics
Wild type and global regulatory O157:H7 mutants (*rpoS, phoP/Q*) will be used to identify virulence genes that are both up and down-regulated in the bovine/ovine
The identification of novel genes involved in the persistence of VTEC in the bovine/ovine

VTEC O157:H7 strain characterisation

Project leader: Bacteriology Department, VLA Weybridge
Funding: Mixed internal and external
Quantitative Hep-2 tissue culture adhesion assay and Fluorescent-actin staining (FAS) test
ELISA/Western blot to determine levels of intimin expression
Acid tolerance at pH 2.5 for 2hrs
Detailed characterisation of VTEC strains causing calf dysentery

EHEC O157 pathogenesis: ovine and animal model studies

Project leader: Prof. M.J. Woodward
Collaborators: Dr G. Pearson and Mr A. Wales, University of Bristol Veterinary School
Funding: MAFF-funded project (1999-2002)
To undertake ovine pathogenesis studies with EHEC O157
To develop an animal model and in vitro surrogate model to test the pathogenic potential of O157 strains.
To develop a simple animal model to support studies into the molecular basis of persistence of O157 in farm animals.
The identification of novel adherence mechanisms in non-O157 VTEC

Project Lead: Prof. M.J. Woodward
Collaborators: CAMR, Porton Down
To identify and characterise the mechanism by which non-O157 VTEC, lacking the eae determinant, adhere to epithelial cells

G Paiba, VLA, Weybridge.
Poster presentation:

Verotoxin sequences in Scottish *E.coli* O157 strains

N Turner, L Duthie, F Thomson-Carter and P Carter

Introduction

The verotoxins (VT1 and VT2) are hexameric molecules composed of a central A enzymatic subunit with close similarities to the plant toxin ricin, surrounded by five B subunits which bind to a cell surface glycolipid receptor. The complex is internalised by receptor mediated endocytosis, and the A subunit is activated and translocated into the cytoplasm, where it inhibits protein synthesis by cleaving ribosomal RNA. The presence of the receptor on endothelial cells is responsible for the manifestations of haemolytic uraemic syndrome (HUS). Regional differences in receptor type and in the affinity of verotoxins for different receptors probably account for the different effects of the toxins in different species. In humans, VT receptors are found on renal endothelium, but expression is increased by stimulation with certain cytokines.

VT2 is more commonly associated with HUS and is more potent when administered systemically to mice. Several VT2 variants have been described. We analyzed the toxin types associated with disease in Scotland, with a view to making recombinant verotoxin.

Methods

Initial studies were undertaken on two *E.coli* O157 strains isolated from patients known to have had HUS. Subsequently, a wider range of bacterial strains was used, from stored samples representing strains involved in several major outbreaks in Scotland, and from sporadic isolates selected to represent different regions and years.

Certain regions of verotoxin genes are commonly used for identifying the presence of VT1 or VT2 genes by PCR, in separate reactions for each toxin type. We were interested in whole gene sequences and therefore used a PCR reaction that amplified the whole VT operon. Products were cleaned up, digested with restriction enzymes and cloned into pUC plasmids, in which individual clones were sequenced using pUC/M13 universal primers and automated fluorescent sequencing (Applied Biosystems).

Later, direct sequencing of polymorphic regions encoding the VTB subunit was undertaken using sequence-specific primers.

Toxin subtypes were expressed singly in DH5α cells after subcloning into pUC plasmids, and crude lysates or supernatants, or partially purified toxin preparations, were used in bioassays.

Candidate bioassays for toxin detection and quantitation were compared. It was concluded that one of the simplest, the neutral red cell viability assay, was the most robust and easily reproducible. The slight increase in sensitivity that could be achieved by assays of protein synthesis was counterbalanced by their greater complexity and reduced reproducibility.

Results

First sequences

Initial observations on bacteria isolated from patients affected in various outbreaks in Scotland, as well as from sporadic examples of the disease, confirmed the close association with VT2 which has previously been documented in Scotland and elsewhere. However, we found that the VT2 genes from the first strains we looked at were of mixed sequence, corresponding to two subtypes of VT2.

These subtypes differ over a small region of the VT2 B gene, including residues that may be important in determining receptor specificity. One sequence corresponded to the ‘classical’ VT2 gene, the other to the well-described variant VT2c. The co-carriage of these two variants in disease-causing strains has been recorded previously.

In a subsequent survey, of some 20 strains from geographically and temporally separated cases, we identified both sequences in all but one.

Bioassays

Comparing their biological effects on Vero and HeLa cells, significant differences between the two toxin subtypes were
observed. Results from DH5α clones expressing single verotoxin types are shown on HeLa and Vero cells. It is not clear whether these translate into clinically relevant differences, but both subtypes must be considered potentially important for further studies.

Discussion

VT2 and VT2c sequences differ extensively over a very small region of the B subunit and infrequently elsewhere; the coding region of the A subunit and most of the remainder of the B subunit are identical. There are nine nucleotide differences and two amino acid differences over 36bp. The evolutionary basis of this is unclear, but its persistence suggests selective pressure. A number of other ‘lesser’ variants have been described, but not isolated with the same frequency as these two. We did not find any in this study of Scottish strains.

It is known that some reagents may identify both VT2 and VT2c, but others may bind to only one of the two. The significance of these strains for tissue activity has been alluded to above; in addition, the positions of the changed residues suggest that they may affect binding to gb3 receptors.

Conclusions

In Scotland, HUS and E.coli O157-related disease are associated with the co-production of two closely related verotoxin 2 variants. It was not possible to identify from our (or from other) epidemiological data whether either variant alone, or both together, were critical for induction of disease. Further analyses must take this complexity into consideration.

Acknowledgements

Funding for the initial studies came from a MRC ROPA award to NT. Additional support has been received from the Renal Research Fund in Aberdeen and from Lothian Hospitals Endowment Trust.

N Turner, Royal Infirmary, Edinburgh; L Duthie; F Thompson-Carter, Scottish Reference Laboratory for E.coli O157 and Campylobacter, Grampian University Hospitals Trust, Aberdeen; P Carter, Department of Medical Microbiology, University of Aberdeen.
### Author contacts:

<table>
<thead>
<tr>
<th>Name</th>
<th>Telephone Number</th>
<th>Fax</th>
<th>E-mail</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr B Adak</td>
<td>020 8200 6868 Ext 4551</td>
<td>020 8200 7868</td>
<td><a href="mailto:badak@phls.co.uk">badak@phls.co.uk</a></td>
</tr>
<tr>
<td>Ms H Aird</td>
<td>01772 710100</td>
<td>01772 713681</td>
<td><a href="mailto:micro@presphls.demon.co.uk">micro@presphls.demon.co.uk</a></td>
</tr>
<tr>
<td>Dr R Chalmers</td>
<td>01222 521997</td>
<td>01222 521987</td>
<td><a href="mailto:rachel.chalmers@phls.wales.nhs.uk">rachel.chalmers@phls.wales.nhs.uk</a></td>
</tr>
<tr>
<td>Mr T Cheasty</td>
<td>020 8200 4400 Ext 3173</td>
<td>020 8905 9929</td>
<td><a href="mailto:tcheasty@phls.nhs.uk">tcheasty@phls.nhs.uk</a></td>
</tr>
<tr>
<td>Dr J Coia</td>
<td>0131 537 1927</td>
<td>0131 537 1024</td>
<td><a href="mailto:john.coia@ed.ac.uk">john.coia@ed.ac.uk</a></td>
</tr>
<tr>
<td>Ms C Currie</td>
<td>0131 650 3136/3134</td>
<td></td>
<td><a href="mailto:cgcurrie@srv1.med.ed.ac.uk">cgcurrie@srv1.med.ed.ac.uk</a></td>
</tr>
<tr>
<td>Dr D Fenlon</td>
<td>01224 711000</td>
<td>01224 711292</td>
<td><a href="mailto:d.fenlon@ab.sac.ac.uk">d.fenlon@ab.sac.ac.uk</a></td>
</tr>
<tr>
<td>Mr I Fisher</td>
<td>020 8200 6868 Ext 4543</td>
<td>020 8200 7868</td>
<td><a href="mailto:IFisher@phls.nhs.uk">IFisher@phls.nhs.uk</a></td>
</tr>
<tr>
<td>Dr J Ritchie</td>
<td>01224 273725</td>
<td>01224 272703</td>
<td><a href="mailto:j.m.hall@abdn.ac.uk">j.m.hall@abdn.ac.uk</a></td>
</tr>
<tr>
<td>Dr L Humpherson</td>
<td>01292 520331</td>
<td>01292 525071</td>
<td><a href="mailto:L.Humpheson@au.sac.ac.uk">L.Humpheson@au.sac.ac.uk</a></td>
</tr>
<tr>
<td>Dr J Huntley</td>
<td>0131 445 5111</td>
<td>0131 445 6111</td>
<td><a href="mailto:huntj@mri.sari.ac.uk">huntj@mri.sari.ac.uk</a></td>
</tr>
<tr>
<td>Dr B Keevil</td>
<td>01980 612310</td>
<td>01980 611096</td>
<td><a href="mailto:bill.keevil@camr.org.uk">bill.keevil@camr.org.uk</a></td>
</tr>
<tr>
<td>Ms M Locking</td>
<td>0141 300 1118</td>
<td>0141 300 1170</td>
<td><a href="mailto:Mary.locking@scieh.csa.scot.nhs.uk">Mary.locking@scieh.csa.scot.nhs.uk</a></td>
</tr>
<tr>
<td>Dr S O’Brien</td>
<td>020 8200 6868 Ext 4422</td>
<td>020 8200 7868</td>
<td><a href="mailto:SOBrien@phls.nhs.uk">SOBrien@phls.nhs.uk</a></td>
</tr>
<tr>
<td>Dr I Ogden</td>
<td>01224 551132</td>
<td>01224 685604</td>
<td><a href="mailto:i.ogden@abdn.ac.uk">i.ogden@abdn.ac.uk</a></td>
</tr>
<tr>
<td>Dr G Paiba</td>
<td>01932 357893</td>
<td>01932 349983</td>
<td><a href="mailto:g.a.paiba@vla.maff.gov.uk">g.a.paiba@vla.maff.gov.uk</a></td>
</tr>
<tr>
<td>Dr J Roberts</td>
<td>0171 927 2366</td>
<td>0171 580 8183</td>
<td><a href="mailto:Jenny.Roberts@lshtm.ac.uk">Jenny.Roberts@lshtm.ac.uk</a></td>
</tr>
<tr>
<td>Dr H Smith</td>
<td>020 8200 4400 Ext 3114</td>
<td>020 8905 9929</td>
<td><a href="mailto:hsmith@phls.nhs.uk">hsmith@phls.nhs.uk</a></td>
</tr>
<tr>
<td>Dr C Stewart</td>
<td>01224 712751</td>
<td>01224 716687</td>
<td><a href="mailto:css@rri.sari.ac.uk">css@rri.sari.ac.uk</a></td>
</tr>
<tr>
<td>Mr B Synge</td>
<td>01463 243030</td>
<td>01463 711103</td>
<td><a href="mailto:B.Synge@ed.sac.ac.uk">B.Synge@ed.sac.ac.uk</a></td>
</tr>
<tr>
<td>Dr F Thomson-Carter</td>
<td>01224 553819</td>
<td>01224 840632</td>
<td><a href="mailto:mmb049@abdn.ac.uk">mmb049@abdn.ac.uk</a></td>
</tr>
<tr>
<td>Dr A Todd</td>
<td>01236 748748</td>
<td>01236 760015</td>
<td><a href="mailto:Andrew@todds.demon.co.uk">Andrew@todds.demon.co.uk</a></td>
</tr>
<tr>
<td>Prof N Turner</td>
<td>0131 536 2315</td>
<td>0131 536 1541</td>
<td><a href="mailto:neil.turner@ed.ac.uk">neil.turner@ed.ac.uk</a></td>
</tr>
<tr>
<td>Prof M Woolhouse</td>
<td>0131 650 6277</td>
<td>0131 650 6277</td>
<td><a href="mailto:mark.woolhouse@ed.ac.uk">mark.woolhouse@ed.ac.uk</a></td>
</tr>
<tr>
<td>Name</td>
<td>Organization</td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------------</td>
<td>---------------------------------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dr Bob Adak</td>
<td>CDCS, London</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dr Syed Ahmed</td>
<td>Greater Glasgow Health Board</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ms Heather Aird</td>
<td>PHLS North West (Preston)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mr Derek Armstrong</td>
<td>Meat and Livestock Commission</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dr Dugald Baird</td>
<td>Hairmyres Hospital, East Kilbride</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dr Yvonne Beaton</td>
<td>University of Aberdeen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dr Jim Beattie</td>
<td>Royal Hospital for Sick Children, Glasgow</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prof Eric Bolton</td>
<td>PHLS, Preston</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mrs Kim Bottomley</td>
<td>Grampian Health Board</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dr Hilary Bowyer</td>
<td>Borders Health Board</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dr David Breen</td>
<td>Dumfries and Galloway Health Board</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dr Anthony Breslin</td>
<td>Forth Valley Health Board</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dr Morag Brown</td>
<td>Borders General Hospital</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dr Gemma Browne</td>
<td>Royal Infirmary, Edinburgh</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dr Lynda Browning</td>
<td>SCIEH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dr Jim Bruce</td>
<td>Scottish Agricultural College, Auchincruive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mr Alex Campbell</td>
<td>West Lothian Council</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dr Rachel Chalmers</td>
<td>CDSC, Wales</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mr Tom Cheasty</td>
<td>CDSC, London</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dr John Coia</td>
<td>Western General Hospital, Edinburgh</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mr Ian Connell</td>
<td>Argyll and Clyde Health Board</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dr Paul Cook</td>
<td>Department of Health, London</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ms Elizabeth Corbett</td>
<td>Glasgow City Council</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dr John Cowden</td>
<td>SCIEH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mr Frank Crowe</td>
<td>Crown Office, Edinburgh</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ms Carol Currie</td>
<td>University of Edinburgh</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dr Barry Dale</td>
<td>Dumfries and Galloway Royal Infirmary</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dr Helen Davison</td>
<td>Royal (Dick) School of Veterinary Studies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mr Spencer Dawson</td>
<td>Meat Hygiene Service</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ms Christine Dick</td>
<td>Stirling Council</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dr Willie Donachie</td>
<td>Moredun Research Foundation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dr Martin Donaghy</td>
<td>Scottish Executive, Edinburgh</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dr Pauline Dunlop</td>
<td>SERAD, Perth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dr Giles Edwards</td>
<td>Scottish Salmonella Reference Laboratory</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dr Marianne Ellin</td>
<td>PHLS Sheffield</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mr Juan Escala</td>
<td>State Veterinary Service, Hamilton</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dr David Fenlon</td>
<td>SAC Veterinary Science Division, Aberdeen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prof Julie Fitzpatrick</td>
<td>Glasgow University Veterinary School</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mr Ian Fisher</td>
<td>CDSC, London</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mr Geoff Foster</td>
<td>SAC Veterinary Science Division, Inverness</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dr Andrew Fraser</td>
<td>Scottish Executive, Edinburgh</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dr Nigel French</td>
<td>University of Liverpool</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dr David Gally</td>
<td>University of Edinburgh</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mr Paul Gayford</td>
<td>Ministry of Agriculture, Fisheries and Food</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ms Jean Gilchrist</td>
<td>SERAD, Edinburgh</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mr Nicholas Glover</td>
<td>Aberdeen City Council</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ms Elizabeth Gray</td>
<td>North Lanarkshire Council</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Participants

Mr Arthur Griffiths  State Veterinary Service, Galashiels
Mr George Gunn  SAC Veterinary Science Division, Inverness
Ms Hayley Haining  Glasgow University Veterinary School
Dr Mary Hanson  Western General Hospital, Edinburgh
Dr Robert Hardie  Crosshouse Hospital, Kilmarnock
Dr Judith Hilton  Department of Health, London
Dr Philip Hill  Western Isles Health Board
Ms Mary Howell  Ministry of Agriculture, Fisheries and Food
Dr Lee Humpheson  SAC, Auchincruive
Dr John Huntley  Moreden Research Institute
Mr Gordon Innes  Glasgow City Council
Mr Daniel Johnston  Angus Council
Dr Ian Jones  SCIEH
Mr Jim Kane  Glasgow City Council
Dr Jay Kavi  Warwick Hospital
Dr Bill Keevil  CAMR, Porton Down
Dr Alistair Leanord  Monklands Hospital
Ms Jayne Leith  Grampian Health Board
Dr Kenneth Liddell  Law Hospital, Carluke
Mr Ian Livingstone  Aberdeen City Council
Ms Mary Locking  SCIEH
Dr Chris Low  SAC Veterinary Science Division, Edinburgh
Ms Kirsten McCallum  University of Edinburgh
Ms Pearl Machray  North of Scotland Water
Mr Martin McNab  Inverclyde Council
Ms Marion Macrae  University of Aberdeen
Ms Cath McVicar  SERAD, Edinburgh
Mr Henry Mather  Scottish Salmonella Reference Laboratory
Dr Louise Matthews  Royal (Dick) School of Veterinary Studies
Dr Heather Maxwell  Royal Hospital for Sick Children, Glasgow
Dr Dominic Mellor  Glasgow University Veterinary School
Dr Jim Miller  Lanarkshire Health Board
Dr Robert Mitchell  PHLS, London
Mr Crawford Morgan  West Lothian Council
Mr Grant Naismith  Dumfries and Galloway Council
Dr Stuart Naylor  Moredun Research Foundation
Dr Robert Nelson  Western Infirmary, Glasgow
Ms Jennifer Nimmo  East Dunbartonshire Council
Prof Andrea Nolan  Glasgow University Veterinary School
Dr Ken Oates  Highland Health Board
Dr Sarah O'Brien  CDSC, London
Dr Maire O'Connor  South Eastern Health Board, Kilkenny
Dr Iain Ogden  University of Aberdeen
Dr David Old  Tayside University Hospitals NHS Trust
Dr Giles Paiba  VLA, Weybridge
Dr Nick Parham  Glasgow University Veterinary School
Prof Hugh Pennington  University of Aberdeen Medical School
Participants

Mr Gordon Pollock, Midlothian Council
Mr Kenneth Punter, West of Scotland Water
Mr Philip Rae, University of Edinburgh
Dr Colin Ramsay, SCIEH
Prof Stuart Reid, Glasgow University Veterinary School
Dr Tom Reid, Grampian University NHS Trust
Prof Bill Reilly, SCIEH
Dr Jennifer Ritchie, University of Aberdeen
Dr David Roberts, University of Aberdeen
Dr Jenny Roberts, London School of Hygiene and Tropical Medicine
Prof Mark Roberts, Glasgow University Veterinary School
Dr Carole Ross, SERAD, Edinburgh
Dr Clark Sharp, Edinburgh
Dr Ros Skinner, Scottish Executive, Edinburgh
Mr Alastair Smith, SAC Veterinary Science Division, Inverness
Dr Henry Smith, CDSC, London
Mr David Speirs, Glasgow City Council
Mr Billy Steele, Glasgow University Veterinary School
Ms Fiona Steele, East Dunbartonshire Council
Dr Janet Stevenson, Argyll and Clyde Health Board
Mr Cameron Stewart, SCIEH
Dr Colin Stewart, Rowett Research Institute, Aberdeen
Dr Alastair Sutherland, Glasgow Caledonian University
Mr Burti Synge, SAC Veterinary Services, Inverness
Prof David Taylor, Glasgow University Veterinary School
Ms Helen Tement, SAC Veterinary Science Division, Inverness
Dr Fiona Thomson-Carter, Grampian University Hospitals
Dr Chris Thorns, VLC, Weybridge
Dr Andrew Todd, Monklands Hospital, Lanarkshire
Prof Neil Turner, Royal Infirmary, Edinburgh
Dr Pauline Upton, Lothian Health Board
Dr Bill Uttley, Royal Hospital for Sick Children, Edinburgh
Dr Marianne Vinson, Argyll and Clyde Health Board
Dr Andrew Vinten, SAC, Edinburgh
Dr Alan Williams, Hannah Research Institute
Dr Alun Williams, Glasgow University Veterinary School
Dr Colin Williams, Royal Alexandria Hospital, Paisley
Dr Ian Wilson, Belfast City Hospital
Dr Raymond Wiseman, West Lothian Healthcare NHS Trust
Prof Mark Woolhouse, Royal (Dick) School of Veterinary Studies, Edinburgh
Dr Peter Wright, PHLS, Preston
Ms Lynn Young, SCIEH
Dr Gillian Ysart, SERAD, Edinburgh
Scottish Centre for Infection and Environmental Health,

Clifton House, Clifton Place, Glasgow, G3 7LN, Scotland.
Telephone: 0141 - 300 1100 Fax: 0141 - 300 1172