Verocytotoxigenic *E. coli*

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PREFACE

Verocytotoxin producing *Escherichia coli* (VTEC), and in particular, strains of serogroup O157, have emerged as significant pathogens causing a range of severe and potentially fatal illnesses. The European Union has recognised the threat posed by *E. coli* O157:H7 and the need to devise control strategies based on an understanding of VTEC pathogenicity, transmission, survival and growth. It also acknowledges the importance of informing farmers, veterinarians, food producers and health authorities so that each of these groups can act appropriately to reduce the overall hazards posed by these organisms. To contribute to the development and dissemination of effective control strategies, the European Commission funded a Concerted Action Project "A European study on animal, food, and biomedical aspects of verocytotoxigenic *E. coli* including serotype O157:H7, an emerging pathogen" (CT98-3935) within the Agriculture and Agro-industry Framework IV Research Programme (1998-2001). This book, compiled under the auspices of the above project, integrates contributions from project participants and invited contributors, to provide a comprehensive overview of the current state of research on VTEC. It should be of interest to current workers in this area, and those seeking an effective introduction to research on this important pathogen.

This book, containing contributions from the many and diverse research disciplines currently being brought to bear on VTEC, amply demonstrates the success of the EU project in promoting collaboration among scientists from veterinary, food and biomedical backgrounds from 31 participant groups in 12 European countries. It also includes invited contributions from a wider circle of international research leaders in VTEC research, increasing the benefits to be gained from effective communication of the latest research findings, and the means of their application, to end users working in diverse areas of food safety and public health. The focus provided by the project, and the format and content of this book will enable information on the current state of research and its implications to flow to the widest possible audience, preventing duplication of research efforts, and directing future research in this area. As an effective and widely accessible overview, presenting appropriate dissemination of recommendations for dealing with VTEC in Europe, this book should provide a valuable resource for the many disciplines engaged in combating the public health challenges associated with VTEC.

The nomenclature of verotoxin-producing *E. coli* is a complex issue which is still in a state of flux and there are variations in the nomenclature used in different chapters in the book. A table summarising the nomenclature terms for verocytotoxins is provided in the appendix of this book.

The editors would like to thank all those who have contributed chapters to this text, and/or contributed in other ways to the success of the overall project,
encompassing 5 international conferences, workshops and related activities on methodology, survival and growth characteristics, virulence and pathogenicity factors, epidemiology, and measures for the control of VTEC. Conference proceedings from these meetings have been published and are available on request from the project coordinator, or can be downloaded from the project web site http://www.research.teagasc.ie/vteceurope. In addition, a series of technical booklets, likely to be of particular interest to food industry and public health surveillance personnel, are available. The considerable management and coordination activities necessary for the delivery of this project and derived publications, including this book, were provided by Teagasc, The National Food Centre, Dublin, Ireland.

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GERALDINE DUFFY
PATRICIA GARVEY
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In recent years, \textit{E. coli} O157:H7 has achieved considerable notoriety status, seizing public, government and scientific attention. Such a reputation may be justified, considering the abilities of this organism to survive in many environments, including some widely used preservation systems, its low infective dose, the nature of the populations most susceptible to its attack, and the severity and long term nature of its clinical consequences among such groups. The importance of this organism, and the extent to which it is widely well recognised within the public psyche, has prompted considerable concerns among consumers and legislature alike, leading to demands for effective action at all relevant points of the food chain and beyond. Such concerns have spawned considerable inter-sectoral, interagency, and international collaboration, leading to the acquisition of large amounts of valuable information on the nature of this notorious pathogen and the increasing application of this information in the development of effective means to prevent or ameliorate human infection with \textit{E. coli} O157:H7. Thus recent years have seen considerable progress in relation to methodologies, survival characteristics, pathogenic and virulence traits, control measures and epidemiology of VTEC. Much of this progress is usefully reviewed and set in context within other chapters of this book.
There may, however, be a series of more general lessons to be learned from such studies, and from the wider appreciation of *E. coli* O157:H7 as a model emerging pathogen, rather than as a unique adversary. If such a virulent pathogen can emerge from what is arguably the best known and intensively investigated group of human and animal commensals (14) — the most extensively “domesticated” and investigated “genetic test bed” — it is perhaps time to reconsider our relationships with bacteria.

Throughout history, pandemic bacterial infections have modulated the course of human and animal history and evolution, and despite the brief false dawn presented by antibiotic therapy around the middle of the 20th century, it is now increasingly clear that this pattern is likely to continue. If anything, the declining efficacy of antibiotic therapy is a “side show” to the relentless emergence and re-emergence of a series of infective agents capable of causing debilitating and/or fatal conditions in humans, mammals and other life forms (4).

There are nearly as many bacteria in the human intestine as there are cells in the human body, and it is clear that bacteria instigate and modulate many aspects of human physiology, particularly aspects of gut physiology. It is clear that the generally commensal human gut flora, and its rarer pathogenic derivatives, including *E. coli* O157:H7 has been adapting to, competing within, and modulating the gut and gut flora for millennia. Such extended co-evolution is normally considered to favour commensalism, mutualism and/or nonlethal parasitism, as host death is an unfavourable outcome for the parasite (9). Thus, the emergence of life-threatening pathogenic strains or clones could be viewed as aberrant, or at the very least, not to the longer term advantage of the infecting clone. Examination of the emergence of such organisms as *E. coli* O157:H7 may well provide a number of important insights into wider host-parasite interactions, provide pointers as to the mechanisms and future implications of such evolving relationships, and suggest ways of gaining advantage in our interactions with current and emerging pathogens.

*Escherichia coli* O157:H7 was first implicated in infectious disease in 1982 (17) and is now recognised as a major cause of food/water borne illness in the developed world. It is a newly evolved serotype of *E. coli* which has become pathogenic through the acquisition of a number of virulence factors. But what were the processes which provided this clone with such a strong set of “trump card” characteristics, capable on occasion of avoiding or negating the human defence system and what can this process tell us about the possible emergence of similar pathogens in the future?

Genetic analysis has shown that *E. coli* O157:H7 is clonally distinct from other verocytotoxin (VT)-producing serotypes but closely related to serotype O55:H7, a non VT-producing clone associated with infantile diarrhoea. Serotype O55:H7 has some pathogenic characteristics, i.e., it has the intimin gene, but most strains do not usually posses the EPEC plasmid. Serotype O55:H7 is
EMERGENCE OF VEROCYTOTOXIGENIC *E. coli*

reported to have acquired the capability for producing VT and enterohaemolysin via horizontal genetic transfer from other pathogens (23). Acquisition of a new serogroup antigen (O157) led to the emergence of a new and highly virulent pathogen (*E. coli* O157:H7). It has been reported by Bilge et al. (2) that the acquisition of the O157 antigen resulted from a lateral transfer of an rfb region containing the *rfbE* gene. Such exchanges, in the microbial equivalent of a molecular "car boot sale" starkly demonstrate the fluidity of exchange of genetic materials within and beyond the procaryotic world, and confirm the inadvisability of viewing groups of bacteria as "species" within the classical meaning of that term. The emergence of *E. coli* O157:H7 is a clear and unfortunate demonstration that bacterial "species" should be viewed in temporal and temporary terms, i.e., as sets of associated genes, gaining and losing individual characteristics in response to, as well as independently of, environmental stimuli. Perhaps a more accurate analogy is of a football team, where "star" players are bought, sold, traded, benched, and/or dropped within overall club activities! Thus we should not be surprised if, in the future, other currently commensal or opportunist organisms put together a "winning" team, and emerge suddenly and unexpectedly into the "premier league" of human pathogens.

*E. coli* O157:H7 is an unusual pathogen in terms of the severity of disease which it causes. The traditional view of evolution among pathogens was that as they evolve, pathogenicity/virulence decreased so as to ensure survival of the host population. More recent models, however, have disregarded the importance of the relationship between host and pathogen and suggest that the evolution of virulence is dependent on the relationship between the parameters of infection and the transmission process. For example, it has been suggested that the induction of diarrhoea by an enteric pathogen may increase the probability of transmission to new susceptible hosts (9). More recent models, however, paid less attention to the "endgame" of infection, i.e., the abilities of the pathogen to evade or overcome host defence, and places more emphasis on the wider parameters of the cycling of pathogen from the host into the environment, survival and transmission within the environment and access to new host systems. For example, it has been suggested that the induction of diarrhoea by an enteric pathogen may increase the probability of transmission to new susceptible hosts (9). Similarly, increased evolutionary durability and/or ability to modulate metabolism in response to environmental signals allow food/water borne pathogens to successfully survive in the distinctly different environs of the external environment (water, soil, etc.), food production and the host gut. Some of these evolutionary traits can be seen in *E. coli* O157:H7. The initial site of attachment for this pathogen in cattle is in the rumen (3). The extent and results of fermentation in the rumen present an acid environment, applying a selective pressure for the development of acid tolerance in *E. coli* O157:H7 (1, 5). In wider environmental terms, acid rain derived from air pollution with sulphur
dioxide and oxides of nitrogen may have lowered the pH of many environments (water, soil, etc.) creating selective pressures which favours the survival of acid tolerant bacteria. Such acid tolerance will, however, also enhance the survival of pathogens in low pH foods, and will increase the numbers of organisms surviving host defences (gastric acid), effectively reducing the infective dose necessary to cause disease (23). The continuing impact of such selective pressures may lead to the emergence of other acid tolerant bacteria with enhanced resistance characteristics and virulence potential.

While most emphasis on the genetic mobility underlying the emergence of \textit{E. coli} O157:H7 has correctly focused on its acquisition of a highly effective set of pathogenic/virulence characteristics, some other characteristics have been lost. One important step on the evolutionary process from \textit{E. coli} O55:H7 to \textit{E. coli} O157:H7 involved loss of the abilities to produce the enzyme \(\beta\)-glucuronidase (GUD), and to ferment sorbitol. These two phenotypic characteristics have been exploited in the development of selective agars for detection of this serotype.

It is important to recognise \textit{E. coli} O157:H7 as the current manifestation of a set of genes, and that the genetic processes which led to this particular format are continuing. Such plasticity and mobility is clearly demonstrated by the verocytotoxins of VTEC. These toxins, important because of their effects in inhibiting protein synthesis within eucaryotic cells, are already known to occur in a number of forms, i.e., VT1, VT2 and VT2 variants, and other forms, perhaps with significantly different characteristics will continue to emerge in the future. Such developments have significant implications for the future detection, recognition and remediation of verocytotoxins in \textit{E. coli} O157:H7 and related strains. As well as such plasticity within clones, it is important to recognise the impact of horizontal evolution in the mobility of vt genes. These toxins are encoded by lambda-like phage and under laboratory conditions they have been transferred to non-toxigenic strains. These VT-encoding phages are potentially capable of disseminating the ability to produce toxin to other \textit{E. coli} strains and indeed to other bacterial species as evidenced by the detection of vt2 in \textit{Citrobacter freundii} strains isolated from diarrhoeal samples (19). Such distribution of pathogenic genes, presents a potent means for the sudden and probably unexpected emergence of newly pathogenic bacteria. Thus the emergence of \textit{E. coli} O157:H7 may be clinically unfortunate, but is not unusual. Its significance is that it is one of the first demonstrations of the wider implications of gene evolution and horizontal gene among bacteria, reinforcing the need for greater understanding of the patterns of development and movement of such materials, to enable effective interventions and therapies.

While VT-encoding phages have been induced \textit{in vitro} from a number of VTEC strains and the induced phages used to infect other \textit{E. coli} (18), the conditions for phage and other virulence factor transmission \textit{in vivo} have yet to be established. In general, phage induction can be triggered by various forms of
stress, such as exposure to UV light or chemical compounds such as antibiotics, conditions which induce the SOS response. It is possible that many of the processes that are routinely used to kill vegetative bacterial cells inadvertently promote phage induction and release. One procedure under investigation for its potential role in the dissemination of vt genes, is the use of sub-therapeutic levels of antibiotics in animal production (7). With further research into the conditions that trigger the processes of horizontal gene transfer, it may be possible to minimise the emergence of new pathogens through the development of intervention strategies that diminish this risk.

The location of the toxin genes within bacteriophage genomes has proven unfortunate in another respect. In some instances, the expression of the verocytotoxin genes have been shown to be linked to late phage gene expression and thus to the induction of the lytic cycle (22). The administration of antibiotic therapy has sometimes exacerbated patient symptoms through the induction of the phage lytic cycle and the concomitant increase in phage and toxin gene expression (21).

The location of virulence associated factors on mobile genetic elements has implications for the survival and persistence of VTEC strains in the environment. It has been documented that phages in general can survive harsh conditions that are capable of eliminating bacterial populations (10). VT-encoding phage have specifically been shown to be more resistant to exposure to environmental conditions, and to chlorination and pasteurisation, than bacterial cells (12). Bacteriophage are also more efficient vectors for DNA transfer than conjugative plasmids as the process does not require intimate contact between bacterial donor and receptor cells. Thus, DNA important to a population can be preserved until a host for lysogenic conversion is reintroduced in an environmental niche (10).

The emergence of E. coli O157 and other VTEC as important agents of disease in the past twenty years has caused a re-evaluation of our view of pathogens. Horizontal transmission of virulence factors has played a crucial role in the evolution of these strains. It has long been known that the primary known virulence factors of E. coli O157:H7 are associated with transferable DNA elements, and this was recently reaffirmed with the publication of the entire sequence of the chromosome of an E. coli O157:H7 strain (15). The accumulation of virulence factors (phage-encoded vt genes, pathogenicity island-encoded intimin gene, plasmid-encoded enterohaemolysin) through their acquisition on mobile genetic elements has facilitated a very rapid form of evolution. The ability to acquire such virulence genes may result from increased mutation rates and enhanced recombination abilities (11). LeClerc et al. (8) reported that 1% of O157:H7 strains had spontaneous rates of mutation that were 1000 fold higher than those of typical E. coli. This ability of E. coli O157:H7 to hypermutate may
even suggest that the pathogen could acquire new factors that will render it even more virulent and/or persistent.

Comparison of the *E. coli* K12 and *E. coli* O157:H7 genome sequences has also identified numerous other strain specific regions of the *E. coli* O157 genome, encompassing up to a quarter of the genome (15). In these strain specific gene clusters, there are many examples of genes encoding candidate virulence factors and alternative metabolic capacities. Codon usage and base composition analysis, and the identification of remnants of prophages and other mobility elements, again demonstrated extensive genetic exchange, and confirm that the extent of horizontal gene transfer and recombination is far greater than was anticipated. The discovery of these additional DNA segments opens up new avenues of research to investigate the possible roles of these factors in the virulence of *E. coli* O157:H7.

*E. coli* O157:H7 may well be a striking case of such evolutionary changes, but it is not the sole example. Phylogenetic analysis, using sequence data for seven housekeeping genes and for the genes for the major virulence factors of enterohaemorrhagic *E. coli* (EHEC), has demonstrated that *E. coli* O157:H7 and non-O157 VTEC descended from old lineages which acquired similar virulence factors in parallel (16). These authors theorised that many virulence factors had been gained and lost over time in different lineages of pathogenic *E. coli* and reported evidence of recent acquisition of vt genes and the EHEC plasmid, whereas appropriation of the locus of enterocyte effacement (LEE) occurred further in the past. The authors concluded that natural selection favoured an ordered acquisition of genes and the progressive build-up of molecular mechanisms that increased virulence. Thus, the acquisition of a similar collection of virulence factors has permitted a diverse group of *E. coli*, with differing metabolic capacities and environmental tolerances, to produce similar disease when introduced into the human population.

Non-O157 VTEC, in particular serogroups O26, O111, O103 and O145, are increasingly linked to human illness. These serogroups display considerable variation with respect to their complement of virulence factors (20). Variations in gene subtypes, e.g., intimin and vt genes, integration sites for the LEE pathogenicity island, and in plasmid-encoded virulence factor complement, have been identified (13, 20). In some non-O157 VTEC, an additional virulence factor termed high pathogenicity island (HPI) has been identified which was probably also disseminated in clonal VTEC subgroups by horizontal gene transfer (6). Thus it has become clear that VTEC represent a heterogeneous group of strains containing a mosaic pattern of virulence factors. Such a conclusion reinforces the view that ongoing and frequent genetic shuffling and sharing is a routine element of microbial life, and that such processes will continue to generate new or significantly enhanced/modified pathogens in the future.
It is clear that, within the VTEC serogroups, and indeed in the wider group of pathogenic _E. coli_, genetic transfer and evolution is still ongoing. Examination of surveillance data has revealed the chronological emergence and decline of particular clonal lineages, as the acquisition or rearrangement of genetic material gives rise to progressively more successful clones. For example, there are several instances of upsurges in particular O157 phage types which may reflect lysogenic conversion by new phages or genetic rearrangement between different prophages resident on the chromosome. Furthermore, there has been a dramatic rise in the number of reported cases of non-O157 VTEC infection in recent years. While this may derive from an increasing awareness of the role of non-O157 strains in disease and the development of new methods for their detection, it may also reflect the ongoing molecular evolution of such strains by horizontal gene transfer.

In summary, therefore, it is clear that there are important lessons which can and should be learned from the emergence of _E. coli_ O157:H7 as an human pathogen. While much attention has been focused on the specific problems associated with the current symptoms and prognosis of the infections this organism can cause, particularly in relation to at risk and immunocompromised groups, it is important to set the challenges posed by this organism in proper context. The emergence of this pathogen is not a unique, isolated or unlikely to be repeated occurrence. It is more likely to be the first well recognised and investigated representative in an ongoing series of new or significantly modified pathogens which will continue to impact on human health. The particular severity of _E. coli_ O157:H7 infection has galvanised research activity in wider areas of microbial ecology, horizontal gene transfer, and host pathogen interaction and communication, and much of this research will underpin work in the prevention and/or amelioration of infection caused by this particular pathogen. However, in more general terms, many of the advances achieved from such studies should have wider and more strategic application in enabling effective and efficient responses to other, as yet unformed or unidentified pathogens which most surely will continue to emerge to exploit human hosts as one particular aspect of the continuing evolutionary interactions between bacteria and their environment.

REFERENCES


DETECTION OF VEROCYTOTOXIN-PRODUCING 
ESCHERICHIA COLI O157 ON THE FARM 
AND AT THE ABATTOIR 

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INTRODUCTION 

The Need to Investigate Farms and Abattoirs 

Verocytotoxin-producing (VT+) *Escherichia coli* O157 cause a range of symptoms from mild non-bloody diarrhoea to haemorrhagic colitis (HC) and haemolytic-uraemic syndrome (HUS). In the first documented outbreak of HC caused by *E. coli* O157 (53), which occurred in the Northwest USA in 1982, there was a strong association between infection and prior consumption of ground beef. Reported outbreaks of *E. coli* O157 infection have often been very severe, with high mortality rates, particularly in the elderly. Such outbreaks, following consumption of undercooked beef, occurred in Ontario, Canada, in September 1985, in which 17 of 55 affected residents died (7) and in Lanarkshire, Scotland, in November and December 1996, which resulted in 20 deaths among the 496 people affected. Many other outbreaks world-wide have implicated foods of animal origin, or food or water contaminated with animal manure, as a source of infection. Because of the potential severity of the infection and the steadily rising incidence of infection in many countries, there is often a need to trace a source of infection in an outbreak, or to perform surveillance of the animal population, in order to elucidate the epidemiology and ecology of this organism and thereby enable appropriate intervention measures to be put in place.

Animals Carrying *E. coli* O157 and Other VTEC 

Strains of *E. coli* first isolated from diarrhoeal cattle in Argentina in 1977 were later shown to be VT+ *E. coli* O157 (45); these are probably the first documented isolates of the organism from cattle. *E. coli* O157 have also been isolated from healthy cattle, sampled during investigations of possible sources
of human infections or at random. The location, animal population, prevalence of *E. coli* O157 and the isolation methods used are summarised in Tables 1 and 2, respectively.

**TABLE 1.**
SURVEYS OF CATTLE POSSIBLY IMPLICATED IN OUTBREAKS OF *E. coli* O157 INFECTION. SMAC, DIRECT CULTURE ON SORBITOL MACCONKEY AGAR; MTSB-SMAC, ENRICHMENT CULTURE IN MODIFIED TRYPOTNE SOYA BROTH AND SUBCULTURE TO SMAC; CRSMAC, DIRECT CULTURE ON SMAC SUPPLEMENTED WITH CEFIXIME AND RHAMNOSE; IMS/CTSMAC, ENRICHMENT CULTURE IN BUFFERED PEPTONE WATER FOLLOWED BY IMMUNOMAGNETIC SEPARATION AND CULTURE ON SMAC SUPPLEMENTED WITH CEFIXIME AND TELLURITE.

<table>
<thead>
<tr>
<th>Year</th>
<th>Location</th>
<th>Type of cattle</th>
<th>Place of sampling</th>
<th>Method used</th>
<th>% positive</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1991</td>
<td>Wisconsin, USA</td>
<td>Dairy</td>
<td>Farm</td>
<td>MTSB/SMAC</td>
<td>0</td>
<td>70</td>
</tr>
<tr>
<td>1992</td>
<td>Sheffield, UK</td>
<td>Mixed</td>
<td>Abattoir</td>
<td>CRSMAC</td>
<td>3.9</td>
<td>15</td>
</tr>
<tr>
<td>1992</td>
<td>Scotland</td>
<td>Calves</td>
<td>Routine submissions</td>
<td>SMAC</td>
<td>0.4</td>
<td>58</td>
</tr>
<tr>
<td>1993</td>
<td>Sheffield, UK</td>
<td>Dairy</td>
<td>Farm</td>
<td>IMS/CTSMAC</td>
<td>9.5</td>
<td>42</td>
</tr>
<tr>
<td>1993</td>
<td>Galicia, Spain</td>
<td>Calves</td>
<td>Farm</td>
<td>SMAC</td>
<td>0.5</td>
<td>3</td>
</tr>
</tbody>
</table>

**TABLE 2.**
SURVEYS OF CATTLE SAMPLED AT RANDOM FOR THE PRESENCE OF *E. coli* O157. SMAC, MTSB/SMAC AND IMS/CTSMAC, AS TABLE 1.

<table>
<thead>
<tr>
<th>Year</th>
<th>Location</th>
<th>Type of cattle</th>
<th>Place of sampling</th>
<th>Method used</th>
<th>% positive</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1987</td>
<td>Sheffield, UK</td>
<td>Mixed</td>
<td>Abattoir</td>
<td>SMAC</td>
<td>0.9</td>
<td>17</td>
</tr>
<tr>
<td>1990</td>
<td>Berlin, Germany</td>
<td>Beef</td>
<td>Abattoir</td>
<td>SMAC</td>
<td>0.8</td>
<td>43</td>
</tr>
<tr>
<td>1991</td>
<td>Wisconsin, USA</td>
<td>Mixed</td>
<td>Farm</td>
<td>mTSB/SMAC</td>
<td>0.4</td>
<td>70</td>
</tr>
<tr>
<td>1993</td>
<td>Galicia, Spain</td>
<td>Calves</td>
<td>Farm</td>
<td>SMAC</td>
<td>1.8</td>
<td>3</td>
</tr>
<tr>
<td>1993</td>
<td>Washington State, USA</td>
<td>Dairy</td>
<td>Farm</td>
<td>SMAC</td>
<td>0.3</td>
<td>27</td>
</tr>
<tr>
<td>1993</td>
<td></td>
<td>Beef</td>
<td>Farm</td>
<td>SMAC</td>
<td>0.7</td>
<td>7</td>
</tr>
<tr>
<td>1999</td>
<td>Brazil</td>
<td>Mixed</td>
<td>Farm/abattoir</td>
<td>CTSMAC</td>
<td>1.5</td>
<td>8</td>
</tr>
</tbody>
</table>

Various factors may affect the prevalence of detection of *E. coli* O157 in cattle. The method of isolation used has a major impact. We have shown that immunomagnetic separation (IMS) increases the sensitivity of detection of *E.*
coli O157 in bovine faeces by 10- to 100-fold (18), and it is apparent from Tables 1 and 2 that prevalence studies which have used IMS have usually reported higher prevalence rates. The geographical area also has an effect, with the organism being apparently more prevalent in the cattle population in Northwestern USA and in Sheffield than in many other areas. The season of the study has a marked influence on the results. In studies of both dairy cattle and beef cattle in Sheffield, we have shown carriage rates of the organism to be consistently much higher in the summer (14,42). Young animals also tend to carry the organism more frequently than older animals (42) and carriage may be affected by diet (27), with animals which are fed grain silage tending to carry the organism more frequently.

Various other animals, particularly ruminants, have been shown to be reservoirs or vectors of E. coli O157 and the location, animal population, prevalence of E. coli O157 and the isolation methods used in these studies are summarised in Table 3.

<table>
<thead>
<tr>
<th>Year</th>
<th>Location</th>
<th>Type of cattle</th>
<th>Place of sampling</th>
<th>Method used</th>
<th>% positive</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1996</td>
<td>Sheffield, UK</td>
<td>Sheep</td>
<td>Abattoir</td>
<td>IMS/CTSMAC</td>
<td>2.2</td>
<td>14</td>
</tr>
<tr>
<td>1996</td>
<td>Idaho, USA</td>
<td>Sheep</td>
<td>Farm</td>
<td>mTSB/CTSMAC</td>
<td>6.2</td>
<td>38</td>
</tr>
<tr>
<td>1996</td>
<td>Cornwall, UK</td>
<td>Dog</td>
<td>Farm</td>
<td>IMS/CTSMAC</td>
<td>-</td>
<td>64</td>
</tr>
<tr>
<td>1997</td>
<td>Sheffield, UK</td>
<td>Goats, sheep and pigs</td>
<td>Open Farm</td>
<td>IMS/CTSMAC</td>
<td>60.0</td>
<td>12</td>
</tr>
<tr>
<td>1997</td>
<td>Sheffield, UK</td>
<td>Deer</td>
<td>Farm</td>
<td>IMS/CTSMAC</td>
<td>33.0</td>
<td>10</td>
</tr>
<tr>
<td>1999</td>
<td>Netherlands</td>
<td>Pigs</td>
<td>Abattoir</td>
<td>IMS/CTSMAC</td>
<td>1.4</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Turkeys</td>
<td>Abattoir</td>
<td>IMS/CTSMAC</td>
<td>1.3</td>
<td></td>
</tr>
</tbody>
</table>

**ON FARM SAMPLING**

Collection of Samples

Direct transmission of E. coli O157 from farm animals to man has been reported on several occasions, either by direct contact with animals (51,52,59) or by contact with animal manure (19). It is important that all appropriate safety procedures (23,29,31) are followed to avoid the risk of infection to sampling personnel.
Collection of Rectal Faeces

Ideally, samples of rectal faeces should be collected from individual animals. For convenience, samples of rectal faeces are most easily collected by means of rectal swabs. We have found that standard swabs used for this purpose usually obtain a faecal sample of about 0.4 to 0.5 g and we have used these effectively in a number of investigations (14-17). However, a slightly larger number of positive animals may be detected if the amount of faeces examined is increased to 1 g or more (54), although such samples are more laborious and costly to collect and examine.

The number of samples that would need to be collected to ensure detection of a positive animal in a herd is influenced by: (1) the herd size or population size to be sampled; (2) the within-herd prevalence of the organism sought; and (3) the statistical confidence limit required for the number of positive samples detected. These factors have been reviewed by Cannon and Roe (6). Table 4 is modified from that of Cannon and Roe (6) and shows the number of animal faecal samples that would be needed to detect *E. coli* O157 in a herd within 95% confidence limits; herd sizes up to 200 head are shown and expected prevalences (based on tables 1-3) in the range of 1% to 20%.

Collection of Faecal Pat, Manure and Manure Slurry Samples

Collection of faecal samples per rectum from farm animals needs the services of a qualified veterinarian. Collection of apparently fresh faecal pat specimens from the farm environment may provide a more convenient and less costly means of obtaining samples. However, obtaining a statistically valid number of samples is much more problematic as due allowance has to be made for the fact that several faecal pats may have been produced by the same animal; opinions differ as to the best approach to adopt to this and statistical advice should be sought in individual cases.

Studies of the survival of *E. coli* O157 in bovine faeces have shown that the organism usually survives for longer periods at lower temperatures and in moist conditions. Under laboratory conditions the organism can survive for 70-100 days at 4-8°C (4,26,67). However, the organism survives for much shorter periods if subject to drying or a higher temperature or if contained in bovine faeces applied to grassland (4). Samples should therefore be taken only from faecal pats which are apparently fresh. We have isolated *E. coli* O157 from manure slurry taken from a dairy farm (16) and Kudva et al. (37) have shown that the organism may survive in slurry for up to 6 weeks. To maximise the chance of recovery of the organism, all samples should be apparently fresh when collected and should be refrigerated during transport to the laboratory.
### TABLE 4.
NUMBER OF FAECAL SAMPLES THAT NEED TO BE EXAMINED TO ENSURE DETECTION OF A POSITIVE ANIMAL WITHIN A GIVEN HERD SIZE (95% CONFIDENCE LIMITS — MODIFIED FROM CANNON AND ROE (1982)).

<table>
<thead>
<tr>
<th>Herd size</th>
<th>Number of samples needed to detect a positive animal the within-herd prevalence is</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20%</td>
</tr>
<tr>
<td>10</td>
<td>8</td>
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<tr>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>30</td>
<td>11</td>
</tr>
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<td>40</td>
<td>12</td>
</tr>
<tr>
<td>50</td>
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<td>60</td>
<td>12</td>
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<tr>
<td>70</td>
<td>13</td>
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<td>80</td>
<td>13</td>
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<tr>
<td>90</td>
<td>13</td>
</tr>
<tr>
<td>100</td>
<td>13</td>
</tr>
<tr>
<td>120</td>
<td>13</td>
</tr>
<tr>
<td>140</td>
<td>13</td>
</tr>
<tr>
<td>160</td>
<td>13</td>
</tr>
<tr>
<td>180</td>
<td>13</td>
</tr>
<tr>
<td>200</td>
<td>13</td>
</tr>
</tbody>
</table>

**Collection of Water Samples**

*E. coli* O157 may survive for periods up to 12 weeks in water at low temperatures and, for reasons that are unclear, the organism survives less well in untreated surface water than it does in treated drinking water (50,66). Contaminated water troughs may play an important role in maintenance and dissemination of *E. coli* O157 on farms (56), as they can be a source of recurrent exposure to the organism. Multiplication of the organism in water sediments in summer has also been demonstrated on farms in Washington State, USA (28). Ideally, therefore, water samples (minimum 100 ml) collected from water troughs should include some of the sediment and again should be refrigerated during transport to the laboratory.

**Collection of Milk Samples**

*E. coli* O157 is readily killed by pasteurisation or other heat treatment (21) and unless a pasteurisation failure is suspected there is little to be gained by examining samples of heat-treated milk. In untreated milk the organism survives for at least 14 days at 5-8°C (41,68) but suffers rapid reductions in numbers.
after 4 days at 22°C (68). Milk samples should therefore be fresh and should be refrigerated during transport to the laboratory.

During investigations of an outbreak of *E. coli* O157 infection associated with consumption of untreated milk (16,42) we isolated the organism on several occasions from milk taken from individual animals but consistently failed to isolate the organism from milk taken from the bulk storage tank, probably due to the dilution factor involved in bulk storage. Therefore, whilst milk may be included as part of the sampling regime, it should not be relied upon as the sole specimen during investigation of a possible outbreak of milk-borne infection.

**Collection of Environmental Samples**

Survival of *E. coli* O157 in the environment has been less well studied. Although the organism appears to survive for many weeks on contaminated straw and on common structural surfaces such as wood and breeze block (50) the value of examining such surfaces during on farm investigations remains to be determined. Sampling of the dairy environment was found to be useful in tracing an outbreak of infection linked to pasteurised milk in Scotland (65). In this investigation a pipe which carried milk from the pasteurisation apparatus to the bottling machine and a discarded bottling machine rubber both yielded *E. coli* O157 indistinguishable from the outbreak strain. This investigation emphasised the importance of using sensitive methods as both isolates were obtained only by using IMS.

**SAMPLING AT THE ABATTOIR**

**Collection of Samples**

During collection of samples, procedures to prevent infection of personnel and other procedures for safe working in an abattoir should be followed (30). The sampling methods for rectal faeces referred to above apply equally well to collecting samples in the abattoir. In the abattoir rectal swabs offer the added advantage of being much more rapidly obtained and therefore interfering less with the smooth running of the slaughter line.

**Sampling of Carcasses**

Sampling of carcasses is a problem, as even if the method used is effective in recovering *E. coli* O157, it may damage or otherwise contaminate the carcass being sampled. The numbers of *E. coli*, and presumably *E. coli* O157, on the surface of a carcass decline during the first 24 h of storage (5,69) and it is therefore important that carcasses are sampled and examined as soon as possible.