

Section 1: Introduction

Verocytotoxin-producing *Escherichia coli* (VTEC), also termed Shiga toxin-producing *E. coli* (STEC), represent a collection of over 400 serotypes of *E. coli*.

VTEC are characterised by the possession of bacteriophage mediated *vtx/stx* genes which encode production of a distinct range of toxins termed Verocytotoxins (Shiga toxins).

VTEC are associated with a broad spectrum of syndromes and human diseases.

Since first being identified as a human pathogen in 1982, VTEC O157: H7 has been responsible for numerous large food and water-borne outbreaks of human infection world-wide. It has been proposed that VTEC O157: H7 evolved in a step-wise manner from a strain of Enteropathogenic *E. coli* (EPEC) belonging to serotype O55: H7.

Much of the research and method development to date has focussed on VTEC O157: H7, although attention is now moving towards investigating the role and disease potential of non-O157 VTEC.

The clinical significance of many VTEC serotypes remains unclear.

Certain VTEC serotypes, including strains of O157: H7, are more commonly associated with severe infections in man. This sub-group of VTEC often possesses additional virulence genes, which are often associated with plasmids, and pathogenicity islands possessed by the bacteria. Many of these strains have the ability to cause attaching effacing (A/E) lesions. This sub-group of VTEC are often referred to as Enterohaemorrhagic *E. coli* (EHEC).

This review brings together scientific evidence and expert opinion from around the world.

1.1 BACKGROUND

1.1.1 The organism

Much of our knowledge of bacteria at a molecular and cellular level has been obtained through studies with *Escherichia coli*, which has become the most extensively studied bacterial species, and even today remains at the forefront of research. This bacterium is commonly occurring but is predominantly associated with the intestinal tract of humans and animals, although it is also able to survive in natural waters and soils and on plants. The majority of

E. coli strains exist in the gastrointestinal tract of humans and animals as harmless commensal organisms. However, this genetically diverse species includes a number of intestinal and extraintestinal pathotypes, which have evolved highly efficient and specialised mechanisms of colonisation and pathogenicity which are described in more detail elsewhere (Donnenberg 2002; Scheutz and Strockbine 2005; Baylis *et al.* 2006). These have developed through the acquisition of virulence-associated genes and the adaptation of this bacterium to its changing surroundings, aided by mutations and natural selection. Many of these pathogenicity associated genes are encoded on mobile and accessory genetic elements such as plasmids, bacteriophages, pathogenicity islands (PAIs) and insertion sequences (IS). This exchange of virulence genes between different bacteria has been responsible for the evolution of different bacterial pathotypes and creation of new virulent clones in which horizontal gene transfer plays a major role (Johnson 2002).

Verocytotoxin-producing *E. coli* (VTEC), which are also referred to as Verotoxigenic *E. coli* or Shiga-toxin producing *E. coli* (STEC) comprise over 400 serotypes of *E. coli* (Scheutz and Strockbine 2005). These *E. coli* strains share possession of bacteriophage mediated genes (*vtx/stx*) which encode the production of a distinct range of toxins that were first described by Konowalchuk in 1977 who observed their potent cytotoxicity against Vero cells (Konowalchuk *et al.* 1977). This observation led to these toxins being termed Verotoxins or Verocytotoxins (VT). However, because of the similarity in structure and biological activity between these toxins and the toxin produced by *Shigella dysenteriae* type 1, the terms Shiga toxin (Stx) and Shiga-like toxin (SLT) have also been adopted, although SLT has now been withdrawn. Not surprisingly, these changes in nomenclature and inconsistent use of terminology have caused much confusion, although it is now widely accepted that the terms Stx and VT for the toxins and STEC and VTEC for the bacteria are equivalent terms to describe this group of bacteria. Throughout this review these bacteria and their toxins will be referred to as VTEC and VT, respectively, although STEC and *Stx* may be used in the context of other researchers' studies.

The most important group of pathogenic *E. coli* to emerge as a serious threat to human health are the Enterohaemorrhagic *E. coli* (EHEC) (Kaper 1998). This sub-group of VTEC are often typified by their ability to cause distinct, often serious, clinical manifestations in humans (see Section 4) such as haemorrhagic colitis (HC) and haemolytic uraemic syndrome (HUS). Furthermore, besides their toxin production capability, these bacteria often share the ability to cause attaching and effacing (A/E) lesions on epithelial

cells using similar mechanisms to those found in Enteropathogenic *E. coli* (EPEC). Moreover, EHEC strains typically possess a large (ca 60 MDa) plasmid which encodes additional genes associated with pathogenicity or regulation of pathogenicity associated genes. In the context of human infection, and in terms of their phylogenetic profiles and histopathology, strains belonging to serotype O157: H7 have long been regarded as 'model EHEC', although strains from other serogroups with similar virulence profiles to O157: H7 may also fall within the EHEC pathotype. These include strains belonging to serogroups O111 and O26, which also represent two traditional EPEC serogroups.

One of the interesting aspects of VTEC has been the relatively recent emergence of these pathogens. Since it was first identified in 1982 by Riley *et al.* (1983) as the cause of two outbreaks of gastroenteritis in the USA, the previously 'rare serotype' *E. coli* O157: H7 has been found to be responsible for an increasing number of large food and waterborne outbreaks of HC throughout the world, including the USA, Canada, United Kingdom and Japan (Kaper and O'Brien 1998). Consequently, much attention and subsequent research effort, particularly in the UK and USA, has focussed largely on serotype O157: H7 (H⁻), although cases of HC and severe infection which could be attributed to VTEC possibly occurred as far back as the 1950's (Bettelheim 2000).

Research has provided a better understanding of this group of bacteria, especially serotype O157: H7, and its physiological response such as survival and growth characteristics, mechanisms of pathogenicity, as well as potential sources and natural reservoirs and routes of transmission from natural hosts, particularly ruminants, to man via the food chain and the environment. Furthermore, method development in the past has largely been directed towards the detection and isolation of serogroup O157, although attention has focussed on other serogroups during the past decade. In countries where VTEC O157 appears to be less prevalent than other VTEC serogroups, methods have been developed to screen for a wider number of VTEC serotypes and some of these methods are now being adopted worldwide.

The realisation that VTEC comprise a substantially larger number of *E. coli* serotypes, some of which are reported to be more prevalent than VTEC O157: H7 (H⁻) in some countries, has led to substantial VTEC research during the last 20 years. Consequently, there is now a wealth of scientific literature and published research on VTEC. Despite this, many questions remain unanswered and gaps in knowledge still exist. These need to be filled if we are to understand, not only VTEC, but also other aspects of bacterial

evolution, including the emergence of new pathotypes, mechanisms involved in pathogenesis and host responses, as well as routes of transmission and the exchange of pathogenicity associated genes among bacteria.

1.1.1.1 Evolution of VTEC O157: H7 from EPEC O55: H7

Given that VTEC O157: H7 emerged as a foodborne pathogen as recently as the early 1980s, one of the fundamental questions asked is where did this pathogen come from? What events have led to the evolution of this and other VTEC? And what are the implications for the evolution of other bacterial pathogens? The evolution of VTEC O157: H7 has been the focus of much research activity, some of which is described in the following section.

Past studies (Feng *et al.* 1998) have used multilocus enzyme electrophoresis (MLEE) to assess the clonal relationship between O157: H7 and other VTEC. This technique separates allelic variants of housekeeping enzymes on the basis of differences in the rate of electrophoretic migration, under non-denaturing conditions. Amino acid replacements can influence the electrostatic charge of proteins, resulting in mobility variation. Consequently, mobility variants can be interpreted in terms of alleles at individual gene loci. This technique is useful for conserved enzymes such as malate dehydrogenase where there is a good agreement between electrophoretic mobility and amino acid replacements as determined by sequencing, but less suitable for highly polymorphic enzymes (Whittam 1998).

Whittam *et al.* have used MLEE to determine allelic variation in 20 enzyme encoding genes from 1,300 *E. coli* isolates, including O157: H7 and other EHEC and EPEC strains (Whittam *et al.* 1993). From this study, comparison of allelic variation among 20 enzyme encoding genes detected by MLEE revealed 15 major electrophoretic types (ET), with each corresponding to a bacterial clone. Genetically, the O157: H7 clone was reported to be closely related to the clone of EPEC O55: H7 which is commonly associated with infantile diarrhoea and was the most prominent EPEC serotype in the UK in the 1960's (Bettelheim 1968).

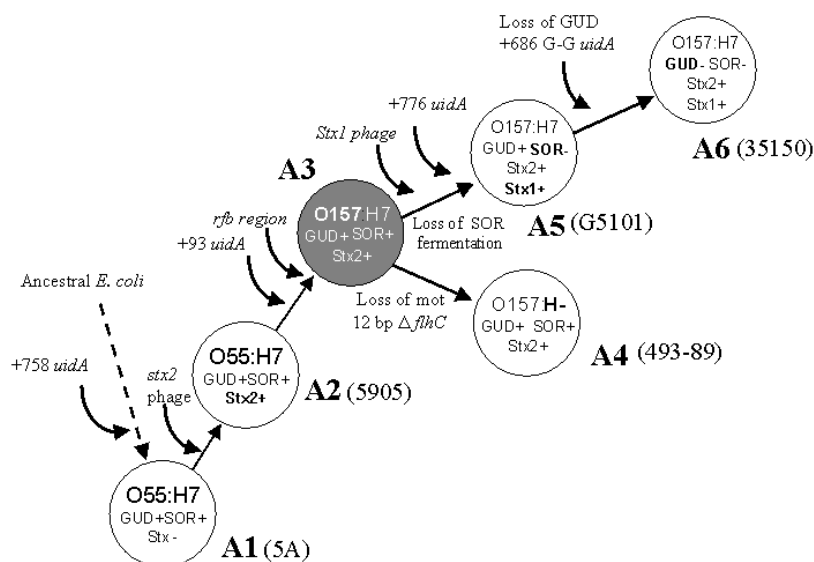
Clonal analysis of 70 strains of EPEC *E. coli* O55 revealed 7 distinct ET's based on allelic variation in 20 enzymes by MLEE (Rodrigues *et al.* 1996). Among these, two major clonal lineages emerged; one comprised typical EPEC, including ET's 1-2 expressing H6 flagella, and the other included O55: H7 strains that were atypical EPEC, with ET5 strains carrying unusual combinations of virulence factors, including some lacking EAF but carrying *eaeA* and another (ET7) that included strains carrying only *eaeA*. It has been

suggested that the ET5 O55: H7 clone, which has the ability to acquire new virulence genes, is the immediate ancestor in the evolution of O157: H7 (Rodrigues *et al.* 1996; Donnenberg and Whittam 2001). The evolution of O157: H7 from O55: H7 is further supported by evidence that the H7 *fliC* genes of O55: H7 and O157: H7 are almost identical (Reid *et al.* 1998).

Comparison of electrophoretic types (ET), multilocus enzyme genotypes and specific genes (*stx1*, *stx2*, *uidA*) or phenotypes (sorbitol fermentation (SF), β -glucuronidase (GUD) activity) has been used by Feng *et al.* (1998) to assess the clonal relationship of O157 strains and the possible evolutionary pathway responsible for the emergence of NSF O157: H7, as well as SF O157: H⁻ strains found in Germany (Feng *et al.* 1998; Whittam 1998). From this data, a stepwise model of the evolutionary emergence of O157: H7 has been proposed (Fig 1.1). The model comprises 6 stages (A1-A6) which starts with an EPEC-like ancestor (A1), similar to the O55: H7 (ET5) described previously. Phenotypically, this ancestral EPEC is assumed to be a SF, GUD positive strain with the -10 mutation in the *uidA* gene and the LEE inserted at *se/C*. In the first stage, this strain acquired *vtx2*, probably via a bacteriophage. This toxigenic O55: H7 (A2) then acquired the +92 mutation in *uidA* gene. In the same stage, lateral transfer of the *rfb* region, which specifies the lipopolysaccharide side chains in Gram negative bacterial somatic O antigens, occurred (Tarr *et al.* 2000). This transfer resulted in the conversion of O55: H7 to O157: H7 (A3). During the conversion of A2 to A3 the strain also acquired the EHEC plasmid (Donnenberg and Whittam 2001). At this point two distinct lineages evolved. One lineage retained the characteristics in A3 (GUD⁺, SF, *Stx2*) but through mutations lost its motility to become O157: H⁻ (A4) which is reported to be present in particular regions of Germany. On the second path, the original A3 strains lost their ability to ferment sorbitol and acquired the *vtx1* gene, probably from a bacteriophage. This intermediate ancestral stage (A5) then lost GUD activity via mutations and an insertion causing a frameshift in the *gusA* (*uidA*) gene (Monday *et al.* 2001) to become the immediate ancestor (A6) of the present day O157: H7 clone.

Subsequent studies support the theory that polymorphisms among distinct sub-populations of *E. coli* O157 are responsible for the emergence of regional subclones (Kim *et al.* 2001). Past analysis of the divergence of 12 genes in *E. coli* O157: H7 shows no evidence of unusually high rates of mutation and molecular evolution in this pathogen (Whittam *et al.* 1998). Instead, insertions or deletions and phage mediated events and recombination probably account for the genome diversity in O157: H7 strains (Perna *et al.* 1998; Kudva *et al.* 2002).

Figure 1.1 Proposed step-wise evolution of *Escherichia coli* O157: H7



Picture courtesy of Dr Peter Feng (US Food and Drug Administration)

1.1.1.1.1 Some historical dates and key developments relating to *Escherichia coli* O157: H7 and VTEC research

1977

- Distinct range of toxins from *E. coli* showing potent cytotoxicity against Vero cells first described by Konowalchuk.

1982

- *E. coli* O157: H7 identified as foodborne human pathogen (causing haemorrhagic colitis) following two ground beef-associated outbreaks in USA.

1983

- World's largest restaurant chain screens beef from suppliers for *Salmonella* and generic *E. coli* and eliminates those suppliers not able to comply with microbiological specifications.

1984

- Laboratory studies determined that *E. coli* O157 has no unusual heat tolerance (Doyle and Schoeni 1984).
- Cooking to 155°F (68.3°C) for several seconds shown to kill *E. coli* O157.
- First outbreak of disease due to *E. coli* O145: H⁻ in Japan (100 children affected).

1985

- Outbreak associated with handling manure-encrusted potatoes points to manure as vehicle of VTEC O157: H7.
- Waterborne route for VTEC O157 infection first suggested.

1986

- CDC investigated a farm associated with *E. coli* O157: H7 outbreak in which unpasteurised milk was identified as the vehicle.
- CDC isolates *E. coli* O157: H7 from faeces of cattle and identified cattle as host/carrier of *E. coli* O157: H7.

1987

- First isolation of *E. coli* O157 from cattle in UK (cattle at slaughter in Sheffield UK).
- 1st International symposium on Shiga Toxin (Verocytotoxin)-producing *Escherichia coli* infections (VTEC 87) held in Toronto, Canada.

1989

- Community outbreak involving 243 cases (21 confirmed) of VTEC infection traced to unchlorinated municipal water supply in Missouri, USA.

1992

- Washington State introduced policy of cooking hamburgers to 155°F (68.3°C).

- Laboratory studies determined *E. coli* O157 can survive processing and storage of fermented dry sausage and appears to have unusual acid tolerance (Glass *et al.* 1992).

1993

- Outbreak of >700 cases and 4 deaths associated with Jack-in-the-Box hamburgers, largely in Washington State. Hamburgers grossly undercooked (<140°F; <60°C).
- FDA publishes new Food Code requiring hamburgers to be cooked to internal temperature of 155°F (68.3°C) for 15 sec.
- USDA issues rule requiring safe handling labels for raw meat and poultry products.
- First recorded case of VTEC O157: H7 infection in New Zealand.

1994

- Outbreak of 23 cases associated with dry-cured salami from California.
- USDA requirement for processing dry fermented sausage, the implementation of critical control points that kill *E. coli* O157.
- USDA declares *E. coli* O157: H7 an adulterant of raw ground beef, establishing a zero tolerance.
- USDA initiates end-product testing of raw ground beef for *E. coli* O157.
- 2nd International Symposium on Shiga Toxin (Verocytotoxin)-producing *Escherichia coli* infections, (VTEC 94) 27-30 June, Bergamo, Italy.

1995

- CDC introduces Foodborne Diseases Active Surveillance Network (FoodNet).
- Discovery and characterisation of the locus for enterocyte effacement (LEE).
- The ACMSF publishes its report on VTEC outlining the recommendations and response of the UK government in relation to VTEC.

- First major outbreak of HUS and bloody diarrhoea in Australia caused by VTEC O111: H- (other VTEC serotypes also isolated). Mettwurst sausage implicated (23 children with HUS).

1996

- Central Scotland outbreak. A total of 378 people affected, 21 deaths. Contaminated meat and poor hygiene practices in a butcher's shop identified as the source.
- USDA publishes rule on Pathogen Reduction and HACCP.
- FDA approves irradiation of red meats.
- Large outbreak of *E. coli* O157: H7 infection caused by contaminated radish sprouts occurs among schoolchildren in Sakai City, Osaka and neighbouring areas in Japan. A total of 10,120 cases, including 12 deaths. Sporadic infections continue in 1997 (948 infections; 714 symptomatic cases and 234 asymptomatic carriers, including 2 deaths).

1997

- CDC FoodNet case-control study reveals that undercooked ground beef was principal food source of *E. coli* O157 infections.
- CDC, FDA, USDA and food industry launched FightBAC program for consumer food safety education.
- USDA increases sample size for testing ground beef for *E. coli* O157.
- Pennington Group reports its findings on the central Scotland outbreak and gives its recommendations relating to *E. coli* O157 research, surveillance, enforcement and prevention of contamination.
- WHO publishes its report on Prevention and Control of EHEC Infections giving recommendations for the control and prevention of VTEC.
- 3rd International Symposium on Shiga Toxin (Verocytotoxin)-producing *Escherichia coli* infections (VTEC 97), 22-26 June, Baltimore, USA.

1998

- USDA requires implementation of HACCP for large meat processing plants.
- USDA publishes key facts document recommending use of thermometer to determine temperature of cooked hamburgers.
- CDC FoodNet results reveal *E. coli* O157 infections increased in 1998 to slightly above 1996 levels.
- Case-control studies by CDC of *E. coli* sporadic cases reveal that visiting a farm, exposure to cattle, and eating undercooked ground beef were three major risk factors for acquiring infection.
- WHO Scientific Working Group publishes its findings concerning current knowledge and recommendations relating to zoonotic non-O157 VTEC.

1999

- USDA introduces more sensitive (IMS) method for detecting *E. coli* O157 in ground beef.
- USDA requires implementation of HACCP for small meat processing plants.
- The 2nd International Symposium of the European Study Group on Enterohaemorrhagic *Escherichia coli* (EHEC), Brussels, Belgium.
- First community outbreak of infections attributable to VTEC O111: H8 reported in the United States. The outbreak involved teenagers attending a cheerleading camp of which 55 became ill (2 with HUS). Illness was associated with consuming salad during the camp's first lunch meal and ice provided in barrels on the camp's final day.

2000

- USDA requires implementation of HACCP for very small meat processing plants.
- USDA approves irradiation of raw beef, pork and lamb.

- First documented outbreak of *Escherichia coli* O157: H7 infection associated with a treated municipal water supply occurs in Walkerton, Ontario, Canada. Overall number of cases exceeded 2,000. *E. coli* O157: H7 confirmed in 167 patients, 65 admitted to hospital, 27 developed HUS and 6 died.
- 4th International Symposium on Shiga Toxin (Verocytotoxin)-producing *Escherichia coli* infections, 29 October - 2 November, Kyoto, Japan.
- Revealed that toxins (VT) are possibly transported in the blood of humans by polymorphonuclear leukocytes.
- Re-emergence of VTEC O103: H2 infections in Brazil (previously isolated from patients in 1986).

2001

- UK Task Force of *E. coli* O157 publishes its final report on *E. coli* O157, identifying known risk factors and potential sources of contamination. The report gives important recommendations to reduce the risk and prevent *E. coli* O157 contamination and infections.
- Complete genome sequence of the Sakai *E. coli* O157: H7 strain (Hayashi *et al.* 2001) and EDL933 hamburger strain (Perna *et al.* 2001) published.
- First reported isolation of EHEC O145: H⁻ from feedlot cattle in Argentina.

2002

- First report of an isolate of VTEC O26: H11 from a patient with HUS in Brazil

2003

- 5th International Symposium on Shiga Toxin (Verocytotoxin)-producing *Escherichia coli* infections, (VTEC 2003) 8-11 June, Edinburgh, Scotland.
- Meat processing industry increases in-house testing for *E. coli* O157 and diverted positive lots to cooked products.
- Some major meat slaughterhouses and processors implement additional food safety interventions such as hide washing and more hygienic hide

removal practices, changes in trim testing and reuse practices, and carcass steam pasteurisation.

- Recto-anal junction in cattle revealed to be a potential region of colonisation and persistence of VTEC O157: H7.

2004

- The first recorded general outbreak caused by VTEC O157 in milk reported in Denmark. The outbreak lasted from September 2003 to March 2004 and was limited to the Greater Copenhagen area. A total of 25 people (18 children and 7 adults) were infected. Dominant symptoms were abdominal cramps and diarrhoea (no HUS).
- The Bioniche (Bioniche Life Sciences Inc., Canada) vaccine against *E. coli* O157: H7 in cattle is developed through a collaboration between the Veterinary Infectious Disease Organization (VIDO) at the University of Saskatchewan in Saskatoon, the University of British Columbia (UBC) in Vancouver, and the Alberta Research Council in Edmonton, Canada.

2005

- The largest outbreak of VTEC O157 ever recorded in Ireland occurred in October/November. Eighteen cases (2 HUS) identified. Person-to-person transmission a major factor in the outbreak, but no microbiological evidence found in water or food.
- In October, 26 cases (13 HUS) of *E. coli* O157: H7 infection associated with a brand of beef burgers in France reported. This was the first community-wide outbreak of *E. coli* O157 to be reported in France.
- Outbreak of VTEC O157 in South Wales, UK associated with contaminated cooked meats supplied to school meal services in September. There were 158 cases (28 reported hospitalisations) across 42 schools. The outbreak resulted in 1 death and the butcher who supplied the meats was jailed for 1 year.
- In Slovenia, a fatal case of bloody diarrhoea caused by VTEC O145 was reported. The 22 month old, a previously healthy girl, died of myocarditis associated with HUS on 8 August.

2006

- 6th International Symposium on Shiga Toxin (Verocytotoxin)-producing *Escherichia coli* infections (VTEC 2006), 29 October – 2 November, Melbourne, Australia.
- Outbreak of HUS in Norway identified between January-March 2006 caused by VTEC O103: H25 (*vtx2a*-positive). A total of 17 cases identified (10 HUS) and 1 fatality. Outbreak traced to cured mutton sausages.
- Outbreak of EHEC O26 (VT1-positive) in Niigata City Japan in August/September 2006. From 2 initial cases identified on 2nd September 2006, a total of 16 cases were later detected although many patients were asymptomatic. Epidemiologically the source of infection was food served in a restaurant on 26th August 2006, although no specific dish could be identified.
- Multi-state VTEC O157: H7 outbreak in the USA in August-September associated with consumption of contaminated bagged Baby Spinach. There were 205 confirmed cases of illness, 103 required hospitalisation (31 HUS) and 3 deaths.
- Bioniche Life Science Inc. *E. coli* O157: H7 cattle vaccine authorized for field use in Canada. The vaccine was developed through a partnership between the University of British Columbia, the Vaccine and Infectious Diseases Organisation (VIDO) at the University of Saskatchewan and the Alberta Research Council.
- The first nationwide outbreak of VTEC O157 in the Netherlands since the start of the enhanced surveillance. Twenty-one confirmed cases were identified (no cases of HUS). Consumption of steak tartare (a beef product that is consumed raw) was the likely cause.

2007

- Outbreak of VTEC O26: H11 occurred in Denmark from February to April 2007. There were 20 laboratory confirmed cases (mainly children) which were linked to the consumption of an organic cured beef sausage.

- Multi-state outbreak of *E. coli* O157 linked to Topp's Brand ground beef patties in September/October 2007. The outbreak involved 8 states, 40 cases of infection (21 known hospitalisations) and two patients developed HUS (no deaths reported). The outbreak resulted in the recall of 21.7 million pounds of frozen ground patties and consequently the Topps Meat Company was forced to close after 67 years in business.
- Outbreak of VTEC O145 and O26 infections in October 2007 associated with consumption of ice cream produced at a farm in Belgium. Five children developed HUS (3 laboratory confirmed cases attributed to O145 and 1 to O26), 7 other individuals (co-exposed) contracted severe diarrhoea. The ice cream was made from pasteurised milk (contamination by food handler suspected).
- VTEC O157 outbreak in the Netherlands in September-October 2007 possibly linked to lettuce/raw vegetables. VTEC O157 (VT1 & VT2-positive) isolated from 36 patients. All had diarrhoea (most had bloody diarrhoea) but no HUS.
- VTEC O157 outbreak in Iceland in September-October 2007. Nine domestically acquired cases (7 hospitalised) and no HUS. Packed lettuce from the Netherlands (identical PFGE pattern to strains from Dutch outbreak described above) suspected but not confirmed.
- The European Food Safety Authority (EFSA) publish the scientific opinion of the Panel on Biological Hazards on monitoring of verotoxigenic *Escherichia coli* (VTEC) and identification of human pathogenic VTEC types.
- The Advisory Committee on the Microbiological Safety of Food (ACMSF) in the UK publishes the "Report on the Safe Cooking of Burgers".

1.1.2 Objectives of this review

This review represents a "stock-take" of past and current research on VTEC/STEC, with particular emphasis on what is currently known about these organisms and the relevance of this information to public health safety and protection. Most importantly, this review identifies gaps in current knowledge on VTEC and related research. This aspect of the review has been greatly assisted by contributions from other VTEC experts worldwide. It is anticipated that this information will enable future research to be more targeted and appropriate. This comprehensive review of past and current literature,

unpublished research and the opinions of other VTEC experts, make this review a valuable source of information for anyone with an interest in VTEC. The information from this review is intended to assist the FSA with future policy decisions on VTEC research and risk management. It is also likely to benefit other Government agencies and enforcement bodies as well as other organisations with an interest in VTEC and food safety research. The up-to-date information contained within this review will greatly assist with the development of appropriate intervention strategies at all stages in the food chain and the implementation of better preventative measures and controls necessary to ensure public health protection in the future.

1.1.3 Scope of this review

This review provides a comprehensive account of VTEC research from past discoveries to current thinking. The scope of this review is broad, covering many aspects of VTEC research from different scientific disciplines. During the last 20 years VTEC, but especially VTEC O157: H7, have been the focus of extensive research and investigation. Consequently, there is now a wealth of information on these bacteria although it is sometimes difficult to put the findings of so much research into context and to appreciate the wider picture, especially the prevalence and public health significance of the VTEC group. This has been made all the more difficult because of the diversity of the research conducted in different countries worldwide. Furthermore, interpreting current evidence from past studies has been made more complicated by the differences in methodology and the approaches used. This has been improved more recently by the development and use of standardised methods, although this applies mainly to VTEC O157: H7 (H⁻). The diversity of VTEC serotypes and strains used in research studies introduces additional variables which can also make comparisons between studies more difficult. This has been made more complicated by the genetic diversity of VTEC and the geographical differences in serotype distribution that have been reported.

In response to the above, where appropriate, this review summarises information derived from different studies to provide an overview of the scientific evidence. Furthermore, whilst this review is comprehensive and every attempt has been made to include as much information on VTEC as possible, there will be published information that is not included or which has been overlooked. Despite the excellent research that has been conducted on VTEC it would be impossible to include information from every published study in this review. Whilst published information from the last 20 years is available, this review has deliberately focussed on research published during

the last 6 years (2000 to 2006) and more recent revelations (2007). However, important scientific discoveries relating to VTEC and many of the key publications from the last 20 years have been included in this review.

1.1.4 Approaches

Information included in this review was obtained from a variety of published and unpublished sources, which are described below. A large proportion of the information was obtained from published research papers and reports in the public domain. Additionally, other VTEC experts and researchers worldwide were consulted and have contributed to this review by providing information from their research as well as their own personal opinions on VTEC and associated research. This important information, and especially the opinions of other VTEC experts, has culminated in a comprehensive review, which has also identified gaps in knowledge and it is these topics that could become the focus of future research activity.

Given the clinical importance of VTEC, especially serotype O157: H7 (H⁻), their wide distribution and the potential risk posed to public health through contaminated food, water and environmental exposure, this review has a deliberately wide scope. Separate sections have been produced on key areas including virulence determinants, methodology, clinical aspects of VTEC infection, epidemiology of VTEC in humans, reservoirs for VTEC in the food chain, and prevention and control of VTEC contamination.

1.1.5 Sources of information on VTEC and related research

Information relating to VTEC research was collated from a wide variety of sources. This included published research and information in the public domain, reports from government and privately funded research in the UK and overseas, information from appropriate web sites and directly from other academic centres and research institutes worldwide.

1.1.5.1 Published information

The amount of published and unpublished information available on these bacteria is substantial and consequently difficult to keep pace with. Books have been published which also give a good account of past research and our current knowledge of these bacteria (Kaper and O'Brien 1998; Duffy *et al.* 2001; Donnenberg 2002). These bacteria, and *E. coli* O157: H7 in particular, have been the subject of numerous published reviews (for example Nataro and Kaper 1998; Paton and Paton 1998; Park *et al.* 1999), although there are

now many other excellent reviews relating to these bacteria or specific subjects that have been the subject of intensive research. Where appropriate, these have been cited and the reader should consult them for more detailed information on a particular subject.

Owing to the importance of these bacteria to the food industry and the potential risk they pose to the consumer, there has been substantial food safety research conducted in recent years. Not all of this research has been specifically targeted at studying these bacteria and researchers have included *E. coli* O157: H7 and other VTEC along with other foodborne pathogens in their studies. Moreover, the role of water, animals and the environment in the transmission of VTEC to man, together with clinical aspects of VTEC and their epidemiology, have all been the focus of much research activity.

Attempts have been made to bring together much of this additional information in this review, although care has been taken to avoid duplication of information where it is deemed unnecessary. Furthermore, some of the studies are specific to a particular country or food commodity and therefore the information emanating from this research is considered to be too narrow for wider applicability. Where appropriate information from these studies has been put into context with what is already known or its relevance elsewhere.

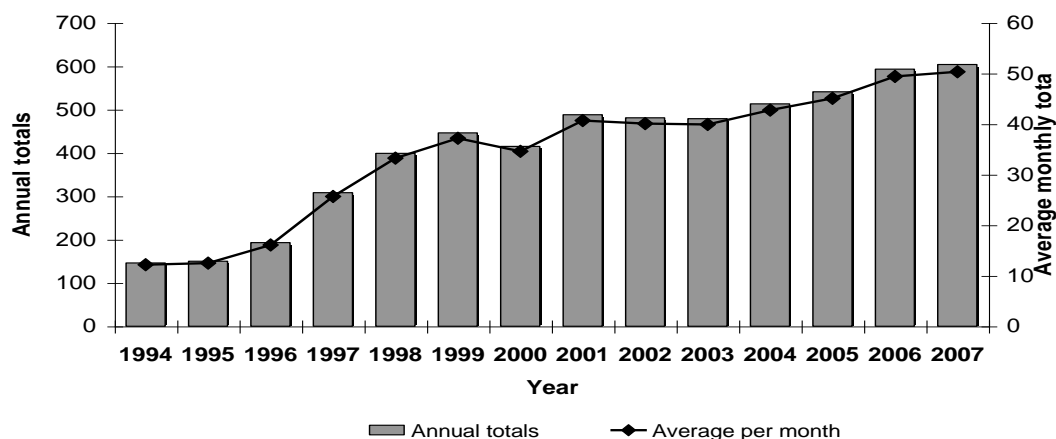
Irrespective of the research conducted, the results, and ultimately, the conclusions of a study, will be heavily dependent on the detection, isolation, confirmation and typing methods used. This is particularly true of studies which have determined the prevalence of VTEC in animals and the environment, including carriage and shedding rates, serotype prevalence and virulence profiles of the strains isolated. Similar studies have been conducted to determine the incidence of VTEC in the food chain, the serotypes involved, the survival characteristics of these bacteria, especially serotype O157: H7, and the effectiveness of processing treatments and intervention strategies. Whilst there are now a number of methods available for detecting VTEC, the majority of standardised procedures, including published reference methods, have been developed for strains belonging to serotype O157: H7. Furthermore, since it was first recognised as a foodborne pathogen in 1982 (Riley *et al.* 1983), methods for the detection of this pathogen have undergone continual development, whilst methods for other VTEC serotypes have developed more slowly and there remains much to do in this area of research. Consequently, the performance of one method can vary enormously from another and this, together with the differences in specificity and sensitivity between methods, can yield different results.

Today there is better standardisation and more consistency between methods used to isolate VTEC. Therefore, results derived from different methods today are likely to be more comparable than those from some of the earlier studies. This is not to say that the results from the early research are any less important. Conversely, much of the information generated during the 1980s was pioneering research which has enabled today's research and our understanding of these bacteria, especially *E. coli* O157: H7, to progress at a phenomenal pace. However, it is important to consider the possible influence that changes in methodology can have on the outcome of research studies and this is true of all aspects of science.

During the past decade publications referring to VTEC/STEC and O157 on PubMed alone have increased to over 500 publications annually (Fig. 1.2). Furthermore, these figures just represent publications that cite these organisms in the title/abstract and which are captured by an on-line search, and only those publications which can be accessed via PubMed. Therefore, the number of research papers in the public domain is likely to be much greater.

Owing to the rapid pace at which VTEC research has been conducted during the past decade and the introduction of new techniques, especially molecular methods, which have greatly improved our understanding of these bacteria, there have been many changes in terminology relating to the organisms, their toxins and other pathogenicity associated factors. Consequently, there remains much confusion, not just outside, but also within, the scientific community. Furthermore, some research can give conflicting information, which then makes it difficult for individuals and Government to implement clear policies which will uphold public health protection or which could be used to plan future research strategies.

Figure 1.2 Published research on PubMed referring to Vero (Shiga) toxin-producing *Escherichia coli* (VTEC/STEC) 1994 to 2007



Source: www.ncbi.nlm.gov/entrez

1.1.5.2 Publicly funded research

In response to the risk to public health and the seriousness of VTEC infection, especially those caused by *E. coli* O157: H7, Governments throughout the world have invested heavily in VTEC research over the years. For further information on Government funding in different countries, the reader should consult the relevant web sites for Government agencies (FSA, FDA, USDA etc) listed in the back of this report.

In the UK, the majority of research funding on pathogenic *E. coli*, including VTEC, comes from the Food Standards Agency (FSA), Biotechnology and Biological Sciences Research Council (BBSRC) and Department for Environment, Food and Rural Affairs (Defra). Research has also been funded by other sources including the Royal Society, Department of Health (DH), the Medical Research Council (MRC) and the Wellcome Trust. The latter was responsible for funding the recent IPRAVE project, which studied the epidemiology and evolution of Enterobacteriaceae infections, including VTEC in humans and domestic animals. The results from this project are discussed in this review. The Microbiological Safety of Food Funders Group (MSFFG) has published two reports on UK publicly funded research relating to VTEC. The first, published in October 1999, gives an overview of publicly funded research relating to VTEC undertaken in the UK during 1990 to 1999. The MSFFG produced a second report, which covers the period 1999 to 2003, and this report published in October 2004 is available from the following address:

http://www.food.gov.uk/multimedia/pdfs/msffg_vtec.PDF

As well as providing details of funding sources and the research undertaken, these reports also identify areas that could benefit from future VTEC research. In addition, the MSFFG maintains a database of projects relating to VTEC research. In their last report a total of 92 projects relating to VTEC research are described (MSFFG 2004). The MSFFG project database is available at <http://www.msffg.org.uk>.

Within Europe, the MSFFG identified over 180 research projects relating to *E. coli* that have been funded by the European Union through the Framework 5 program. A successful project on VTEC to be funded through the EU research programme on Agriculture and Agro-industry (FAIR 1994-1998) was the VTEC Concerted Action Project (CT98-3935). This project “A European study on animal, food, and biomedical aspects of Verocytotoxigenic *E. coli* including serotype O157” brought together VTEC researchers from across the EU, USA and other countries throughout the world, including Australia. The project comprised a series of themed meetings covering all aspects of VTEC research, including methodology, survival and growth, pathogenicity and virulence, control and epidemiology. From each of the 5 meetings a proceedings document was published by Teagasc, The National Food Centre Ireland (now Ashdown Food Centre). Members of the Concerted Action Group and other VTEC experts also produced a comprehensive book on VTEC as part of the output from this project (Duffy *et al.* 2001).

Following on from the success of the VTEC Concerted Action Project (CT98-3935) was the Pathogenic *Escherichia coli* Network (PEN) (www.pen-project.eu). This EU funded co-ordination action project (co-ordinator: Teagasc – Ashdown Food Research Centre) consisted of a network of 35 international research groups from 18 different countries working on pathogenic *E. coli*, including VTEC. Experts from the partner institutions along with other international experts came together at five international conferences, each one devoted to a different aspect of pathogenic *E. coli*.

The key issues addressed by PEN:

1. Methods of detection and molecular characterisation of *E. coli* O157 and other potentially pathogenic strains and serotypes of *E. coli*.
2. Epidemiology and transmission of *E. coli* O157 and other potentially pathogenic strains and serotypes of *E. coli*.
3. Virulence and emerging pathogenic *E. coli*.

4. Ecology of *E. coli* O157 and other potentially pathogenic strains and serotypes of *E. coli*.
5. Control and management of *E. coli* O157 and other potentially pathogenic strains and serotypes of *E. coli*.

In the USA, VTEC research is funded by a number of organisations, including private funding. A comprehensive programme of VTEC research has been funded by the USDA and FDA. The USDA Food Safety and Inspection Service (FSIS) has numerous projects covering many aspects of meat hygiene, risk assessment and control of VTEC (www.fsis.usda.gov). In 2003, the American Meat Industry (AMI) worked with Congress to secure funding on *Listeria monocytogenes* in ready-to-eat meat and poultry products and *E. coli* O157 in raw products. These projects, within the USDA's Agricultural Research Service (ARS), received a \$350,000 increase in annual funding, bringing the total annual budget for this agency to \$2.34 million. The USDA's Co-operative State Research Education and Extension Service (CSREES) also received a \$100,000 increase in annual funding for research projects to control and prevent these pathogens in raw and ready-to-eat products (Anon 2003).

The FDA has a similar programme of VTEC research covering aspects of VTEC relating to food safety (www.cfsan.fda.gov). The Canadian Government has also made a great commitment to VTEC research. In 2001, the Ontario Ministry of Agriculture, Food and Rural Affairs granted approximately \$1.4 million to the private pharmaceutical company Bioniche Life Sciences Inc. to assess the beneficial effects of its *E. coli* O157 cattle vaccine. The company is also contributing \$0.5 million to this project, which is also being sponsored by the Ontario Cattlemen's Association (OCA) (Anon 2001). Since 1997, the OCA has committed more than \$250,000 to *E. coli* specific research and its Research Committee also allocated a further £250,00 to similar research. Projects funded by the OCA include detection methods in beef processing environments (\$21,000) and use of vaccines to reduce faecal shedding (\$24,740). In the US, the American Meat Institute Foundation (AMIF), which is a non-profit research, education and information foundation, established by the American Meat Institute (AMI), has a multi-million dollar per year Food Safety Initiative, which includes *E. coli* O157 research. To date, more than \$3.6 million was raised by more than 120 member companies, non-member companies and other industry groups to fund research programs. Research relating to fresh meat has focussed on *E. coli* O157: H7. Further details of the various research projects, including final reports, is available from the AMIF web site (www.amif.org).

1.1.5.3 Research funded by organisations, institutes and private institutions/companies

As well as Government funding, private organisations have also taken a proactive role and commissioned research funded by their members or by using internal funding. Manufacturers of diagnostic kits have been responsible for the development of a plethora of commercially available methods and kits for the detection, isolation and confirmation of VTEC, particularly *E. coli* O157. In the course of the development of many kits, research will have been conducted, either by the company or by researchers commissioned to perform this work. Information from these studies may be proprietary information, which remains confidential to the company concerned, whereas some companies have published their findings.

1.1.5.4 Additional sources of information

Where appropriate, additional sources of information not already covered by the other categories (published information, publicly funded research and research from organisations, institutes etc) was considered during this review. This included personal opinions and experiences of researchers working on these bacteria.

1.1.5.5 External consultation

The importance of this review is not just in the presentation of information and the summary of past and current VTEC research, but also in the opinions and contributions from other VTEC experts throughout the world. The co-operation and willingness of individuals to share their views and opinions on VTEC and associated research with the project team has culminated in this review, identifying gaps in knowledge and important potential areas for future research.

Given the scope of this review and the specialist nature of many of the topics covered, it was appropriate that this review was subjected to external consultation and review. In addition to the review of the complete document by individuals, with expertise in VTEC research stretching back over many years, where appropriate, specific sections were reviewed by experts with knowledge of a particular topic.

1.1.5.6 Important documents and sources of information on VTEC, including gaps in knowledge

There have been several important documents published which address information on VTEC O157. These include the report of the Advisory Committee on the Microbiological Safety of Food (ACMSF) (1995) the Pennington Group report (1997), which followed the Central Scotland outbreak, The World Health Organisation (WHO) report (1997) "Prevention and Control of EHEC Infections" and the WHO Scientific Working Group (1999) who published their report on Zoonotic non-O157 Shiga toxin-producing *Escherichia coli* (STEC).

In September 2000, under the joint sponsorship of the Food Standards Agency (FSA) Scotland and the Scottish Executive (SE) Health Department, The *E. coli* O157 Task Force was appointed by the Minister for Health and Community Care to address the following:

- review the risk to health of the public in Scotland, and current activities to prevent human infection with *E. coli* O157;
- assess the effectiveness of the present arrangements for co-ordination of action at national and local level; and
- consider what future measures would help protect public health.

In June 2001, the report of the Task Force was published; it identifies various gaps in knowledge at the time of the report. Although some of the information and recommendations in this report have been superseded, it still provides valuable information on VTEC O157, risk factors and recommendations from the Task Force. Copies can still be obtained from The Scottish Executive:

<http://www.scotland.gov.uk/deleted/library3/health/ecoli-00.asp>

and elsewhere, e.g.

<http://www.ecoli-uk.com/Download/ecolitaskfinreport.pdf>

Many of these reports also include useful information on gaps in knowledge which are summarised in Table 1.1. Some of these have now been addressed by subsequent research whereas others remain to a lesser or greater extent gaps in our knowledge of VTEC. Those gaps that have been filled are discussed in Section 8 of this report.

Another important document on VTEC with useful information on these bacteria is the report from AFSSA “Bilan des connaissances relatives aux *Escherichia coli* producteurs de Shiga-toxines (STEC)”, published in April 2003, available at:

<http://www.afssa.fr/Ftp/Afssa/16054-22454.pdf>

Following a request from the European Food Safety Authority (EFSA), the Panel on Biological Hazards (BIOHAZ) was asked to do the following in relation to VTEC:

- a) Identify the strains and/or serotypes of VTEC pathogenic to humans.
- b) Give advice regarding the analytical methods, including testing for virulence factors, to be used to detect and identify the human pathogenic VTEC strains/serotypes from food and animals.
- c) Recommend the monitoring methods in animal populations and foodstuffs that are most optimal from the public health point of view.

In response to this request, EFSA published the following document in 2007:

Scientific Opinion of the Panel on Biological Hazards on a request from EFSA on monitoring of verotoxigenic *Escherichia coli* (VTEC) and identification of human pathogenic VTEC types. *The EFSA Journal* (2007) **579**, 1-61.

This document is available at:

http://www.efsa.europa.eu/EFSA/Scientific_Opinion/biohaz_op_ej579_vtec_en.pdf

Table 1.1 Previously published gaps in knowledge on Verocytotoxin-producing *Escherichia coli* (VTEC)

Gap in Knowledge	Report	Page Number
Research into a) The prevalence/incidence of <i>E. coli</i> O157 in Scottish cattle and other animals and the biology of its carriage. b) To help forecast future incidence/prevalence; and c) To improve the current DNA based methods for its identification	The Pennington Group (1997) Interim report and priority recommendations	2
Research into a) Cooking diced, raw meat heavily contaminated with the strain to check the temperature/time relationship necessary to kill off every single organism through different cooking methods b) How much contact with a contaminated product to cause cross-contamination c) Ways of sanitising carcasses at abattoirs	Determination into the <i>E. coli</i> O157 fatal accident inquiry. Sheriffdom of South Strathclyde, Dumfries & Galloway	15, 39 & 85
Research into The <i>E. coli</i> serogroup O26 e.g. routes of infection and Minimum infectious dose, as these remain unknown.	Molecular Characteristics and Epidemiological Significance of Shiga Toxin-Producing <i>E. coli</i> O26 Strains (Zhang <i>et al.</i> 2000)	2138
Research into <i>E. coli</i> O111: H8	Outbreak amongst teenage campers. http://www.cdc.gov/mmwr/preview/mmwrhtml/mm4915a2.htm	-
Accurate, reliable, "line-side" tests should be developed (simple, rapid, tests that can be used within processing establishments to give an initial indication of contamination).	European Commission SANCO/4320/2001	21

Gap in Knowledge	Report	Page Number
<p>Virulence factors</p> <ul style="list-style-type: none"> • What are the infectious doses of various non-O157 STEC? • Is acid resistance correlated with infectious dose and pathogenicity? • What is the influence of different Shiga toxins on disease pathogenesis? • What factors control toxin regulation <i>in vivo</i>? • What are the mechanisms and sites of toxin adsorption in the intestine? • Understanding of the ecology and mechanisms of transmission of Stx phages is lacking • Where is the site of intestinal colonisation? • What are the mechanisms of LEE-negative colonisation? • Understanding of the variations in LEE is incomplete • What is the role of enterohaemolysins and EspP (i.e. putative virulence factors other than Stx and LEE-mediated factors) in pathogenesis? • How do virulence factors move between bacterial strains? • Further characterisation of virulence factors is required for non-O157 STEC lacking A/E ability and enterohaemolysin production. • How do virulence factors move between strains? <p>Host factors</p> <ul style="list-style-type: none"> • Are anti-toxin antibodies protective in humans? • Are antibodies to other factors protective in animals (e.g. LEE or plasmid-encoded proteins)? • Are there any protective antigens? • What host differences (immune, physiologic, etc) account for variations in age susceptibility? • Does therapy with antibiotics increase the risk of infection (e.g. by removing competitive flora) or the likelihood of developing HUS? • There are many methods for the typing and characterisation of non-O157 STEC. However, apart from serotyping, there is little standardised comparison and exchange of data on non-O157 STEC • Little is known about which foods might be potential vehicles of transmission of non-O157 STEC. 	<p>Zoonotic non-O157 STEC</p> <p>(World Health Organization Scientific Working Group 1999)</p>	<p>4, 13 & 19</p>

Gap in Knowledge	Report	Page Number
<p>Research into:</p> <p>a) The survival and multiplication of <i>E. coli</i> O157 and other VTEC in the environment or on plant surfaces</p> <p>b) Determine whether virulence factors can be transferred to other Enterobacteriaceae such as <i>Citrobacter</i> or <i>Enterobacter</i> spp.</p> <p>c) Investigate the existence of viable, non-culturable forms of <i>E. coli</i> O157: H7 and other EHEC.</p> <p>d) Determine the ability of EHEC to attach to surfaces and form biofilms in the environment and on processing equipment.</p> <p>e) Determine sources and transmission of EHEC contamination of livestock (cattle, sheep, poultry, goats, horses and pigs), so as to target intervention or develop prevention strategies regarding:</p> <ul style="list-style-type: none"> • Survival in water, soil, and feed (including silage), manure/slurry; • Transfer from mother to calf, faeces to hide, and human to animal; • Decontamination of sewage and the use of slurry in agricultural practice <p>f) Determine the susceptibility of EHEC to destruction / washing on different types of animals, e.g. acid washes, pasteurisation, vacuum pasteurisation</p> <p>g) Seed contamination – how do seeds become contaminated? Survival in seeds, growth during hydroponic raising of sprouts. How to decontaminate seeds.</p>	<p>Prevention and control of Enterohaemorrhagic <i>Escherichia coli</i> (EHEC) infections</p> <p>(World Health Organization 1997)</p>	<p>32, 33 & 34</p>
<p>Further research to be carried out to identify why the virulent genes which can cause disease in people are not "switched on" in animals or otherwise do not affect them</p>	<p>Task force on <i>E. coli</i> O157</p> <p>http://www.ecoli-uk.com/Download/ecolitaskfinreport.pdf</p>	<p>24</p>

Gap in Knowledge	Report	Page Number
<p>Research into:</p> <ul style="list-style-type: none"> a) Characterisation of the adhesins of VTEC strains, including the minority that do not produce the characteristic (attaching and effacing) lesions b) <i>In vitro</i> methods for demonstration and detection of pathogenicity determinants to aid laboratory diagnosis c) The development and evaluation of different solid media for O157 VTEC. d) Rapid methods to detect VTEC of all serogroups and Verocytotoxin in food and clinical material e) The development of methods for improved sub-typing of VTEC and particularly O157 VTEC f) The prevalence of O157 VTEC in raw meats, raw cow's milk, cream made from raw cow's milk and raw milk cheeses. g) The relationship between the formulation and colour of cooked minced meat products, the colour of juices, and the temperature achieved and survival of VTEC h) The effect of sanitisers/disinfectants on the survival of VTEC 	<p>ACMSF Report on Verocytotoxin producing <i>Escherichia coli</i> (VTEC)</p> <p>(Advisory Committee on the Microbiological Safety of Food 1995)</p>	<p>1, 4 & 8</p>

In addition to the gaps in knowledge given in Table 1.1, the report of the meeting of the WHO Scientific Working Group in Berlin, Germany, 23-26 June 1998 published their conclusions and identified gaps in knowledge. These were as follows:

- Treatment of severe complications (HC, HUS, TTP) is insufficient and only supportive.
- No preventive measures or interventions in the early stage of disease are presently available.
- Studies evaluating late sequelae are scarce, regional differences cannot be excluded and national trends are unknown.
- How is non-O157 infection related to socio-economic status, occupation and diet?
- Is breast-feeding protective? If so, what is the mechanism?

- What is the role, if any, of diet and competitive exclusion in preventing colonisation and infection?
- Are differences in mucus related to colonisation?
- Are differences in receptor density and receptor type related to disease?
- What is the role of regulatory proteins in disease pathogenesis?
- What is the role of the inflammatory system in disease pathogenesis and contribution to tissue damage?
- The basic public health infrastructure for surveillance for non-O157 VTEC is lacking in most countries.
 - Few countries have reporting systems for VTEC with case definitions that clearly include non-O157.
 - Due to the lack of surveillance systems, variability in data sources and incompleteness of reporting, trends over time have not been determined, outbreaks are unlikely to be recognised and data from various countries cannot easily be compared.
- Information is lacking on the incidence of HUS and on the frequency of isolation of non-O157 VTEC in HUS, diarrhoea and healthy persons.
 - HUS:
 - It has not been documented whether the incidence of HUS has changed since it was first described in 1955.
 - Most countries lack surveillance systems for HUS.
 - Data are lacking on the serotypes of VTEC isolated from persons with HUS in many countries and on temporal trends. These data are especially lacking from developing countries. Data are lacking on the relative importance of various serotypes by country.
 - Data are lacking on the proportion of persons with diarrhoea, caused by various STEC serotypes, who develop HUS.
 - Diarrhoea:
 - Data are lacking on temporal trends in the isolation rate of various VTEC serotypes by country.
 - Data are lacking on the proportion of persons infected with various VTEC serotypes who develop bloody diarrhoea.

- Healthy persons:
 - Additional data on the isolation of non-O157 STEC from healthy persons, by serotype and virulence factors, would be helpful in assessing the pathogenicity of various serotypes. There is a need to look at healthy persons in different environments - urban versus rural areas, for example - because of variation in exposure to animals that may be the major source of non-O157 VTEC.
- Most countries have no recommendations for routine laboratory identification of non-O157 VTEC.
- Animal reservoirs for non-O157 VTEC need to be definitively determined; knowledge of the distribution of non-O157 VTEC in animals, including bovines, is limited on a global scale.
- There is a particular need for more knowledge of the distribution in non-bovine animals of non-O157 VTEC serotypes known to cause human disease.
- The pathogenic potential of the many non-O157 VTEC in animals and foods is yet to be fully defined.
- Knowledge of the incidence of non-O157 VTEC in foods throughout the entire food chain is limited, other than for raw animal products (e.g. meat and milk).
- There is little information on quantification of non-O157 VTEC in foods incriminated in human disease.
- Little is known about which foods might be potential vehicles of transmission of non-O157 VTEC.
- Lack of detailed knowledge about outbreaks has limited our knowledge of the infectious dose of non-O157 VTEC.
- Are there differences between non-O157 and O157 VTEC in terms of specific risk factors for human acquisition?
- Is there significant human-to-human transmission of non-O157 VTEC infection?

- What is the role of water in the transmission of non-O157 VTEC to humans?
- Direct animal-to-human transmission of non-O157 VTEC has not yet been demonstrated.
- The factors that influence the carriage rate and levels of shedding of non-O157 VTEC by food animals are not fully understood.
- Strategies for reducing the carriage rate and levels of shedding of non-O157 VTEC by food animals have not been identified.
- For clinical purposes, reporting the confirmation of VT-production or the presence of *vtx* genes should be the priority.
- For epidemiological and surveillance purposes, reporting should include the following characteristics:
 - Biochemical confirmation as *E. coli* (or other VT-positive organism);
 - O: H serotype;
 - Antimicrobial resistance pattern;
 - Characterisation of other known virulence genes;
 - Further typing information where available.
- The only reliable marker for non-O157 VTEC is VT production or possession of *vtx* genes.
- Several satisfactory screening methods for the detection of non-O157 VTEC have been reported and these are becoming increasingly available as commercial kits.
- Standardised procedures including enrichment are essential for detection and isolation of non-O157 VTEC from foods. Such methods require optimisation because methods developed for O157 VTEC may not be appropriate.
- There are many methods for the typing and characterisation of non-O157 VTEC. However, apart from serotyping, there is little standardised comparison and exchange of data on non-O157 VTEC.

In the UK, the FSA maintains a regular programme committed to VTEC research. In January 2005, during a meeting of VTEC researchers in London, the FSA asked them to prioritise their recommendations for further VTEC research. The following areas of VTEC research were given as areas requiring further research or investigation.

- The transmission of VTEC from the environment to animal and humans and between animals.
- Host specificity in pathogenic *E. coli*.
- Shedding patterns.
- Survival in and responses to the environment.
- Population diversity in O157 and other enteropathogens.
- Reliable primary isolation methods for pathogenic non-O157 VTEC in a variety of sample types (environmental, animal and all food types).
- Reasons why non-O157 (such as O26) 'dominate' in other European countries, USA, Japan, Australia etc., whereas VTEC O157 is most common serotype in UK.
- Determine if there is now an accepted validated sub-typing system for epidemiological typing of VTEC.

This VTEC review will attempt to establish which of the gaps in knowledge identified previously by others have been addressed by research and those that remain. More importantly, this review will describe many of the new discoveries that have been made recently and provide information on further gaps in knowledge that have arisen during the past decade of VTEC research.

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