

Section 8: Summary and Conclusions

8.1 Introduction

This review has achieved two important goals. Firstly, it brings together a wealth of information on VTEC and related topics which cover all aspects of VTEC research. The topics are diverse and include our understanding about the ecology of VTEC, virulence characteristics and pathogenesis, methodology, clinical aspects of VTEC infection, epidemiology, and past, current and future control and intervention strategies. Secondly, it provides information on gaps in knowledge identified in each of the different areas of VTEC research covered within this review.

a) Gaps in knowledge

Research on VTEC, especially *E. coli* O157 : H7, covers a diverse range of topics and disciplines. There is now a wealth of information available on VTEC and research into these bacteria, especially *E. coli* O157: H7, has moved at a tremendous pace over the last decade.

It is inevitable that, with such a fast moving and active area of scientific research, some of the gaps in knowledge identified in this review will have been filled or partially addressed.

However, with further scientific discoveries and a greater understanding of these bacteria, new gaps in knowledge will inevitably become apparent.

What makes this review so valuable is the cooperation and the expert opinion of 66 VTEC researchers from 19 countries. As well as including gaps in knowledge identified in published literature and the public domain, this review includes the personal opinions of researchers, based on their knowledge and their own experience of working with VTEC.

What has become apparent whilst writing this review is that despite the amount of research activity throughout the world and the wealth of available information on VTEC, many unanswered questions still remain. Research to date has answered many questions but it has posed others and it has often revealed how little we still know about this large, diverse group of bacteria.

With each new discovery and with every question answered comes yet more questions.

Some of the main gaps remaining include:

- Alternative mechanisms of attachment/colonisation used by some VTEC (notably non-O157 VTEC)
- Additional virulence associated genes and their role in pathogenesis
- A complete understanding of the pathway of VT (especially VT2 and VT variants) in the human body and their role in HUS
- Non-O157 VTEC
 - Habitats, ecology, routes of transmission to humans
 - Clinical significance
 - Methods for detection and isolation
- The geographical distribution of VTEC serotypes
- Associations between seropathotype and human disease

Although much is now known about VTEC O157: H7 the greatest challenge will be to gain a better understanding of the group, their potential to cause disease and the significance of these bacteria in foods and the environment.

Given the widespread distribution of VTEC the future aims will need to focus on preventing contamination, and the implementation of appropriate intervention strategies and controls in the environment and throughout the food chain. Avoiding contamination in the first place should be the primary objective. As demonstrated in the UK and other countries, preventing contamination of foods by a range of pathogens is also effective against VTEC. This can include:

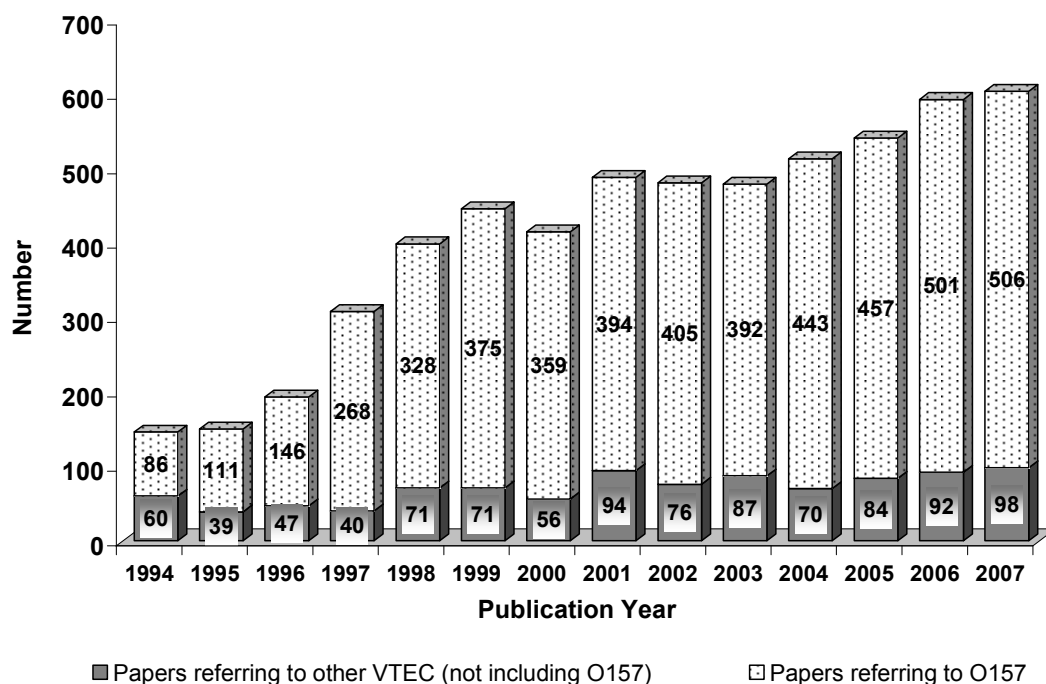
- The adoption of good agricultural practices
- Improved meat hygiene and slaughter practices
- The implementation of HACCP principles throughout the food chain
- Greater awareness of food hygiene and safe handling practices by caterers and the general public
- Better enforcement of food hygiene
- More education of caterers and the general public to increase awareness of food hygiene and safe food handling practices

b) Published VTEC research

Since *E. coli* O157: H7 was first recognised as a foodborne pathogen in 1982 (Riley *et al.* 1983), research on this organism has increased rapidly. In 1982 there were just 3 published papers referring to O157 on PubMed, in 1983 there were 8 and during 1994 there were 86. The number of published

research on O157 gradually increased and during 2001 there were 394 papers relating to *E. coli* O157 on PubMed (Figure 8.1). This annual level of published research has been sustained ever since and from 2006 over 500 published papers referring to O157 were available on PubMed.

Figure 8.1 Published papers on PubMed referring to or including research on *E. coli* O157 and other VTEC or topics relating to the toxins and pathogenicity



This review estimated that over 30 peer-reviewed papers referring to studies with *E. coli* O157, or reports associated with this pathogen, are published each month. Given the number and size of outbreaks attributed to *E. coli* O157: H7 and the serious public health threat posed by this pathogen, much of the attention of Governments and researchers has focussed on this serotype. The number of papers specifically referring to research on non-O157 VTEC or work with the toxins, methods and typing, or disease attributed to VTEC, has also gradually increased. Figure 8.1 gives an indication of the number of papers referring to VTEC, not including *E. coli* O157. However, given the diversity of research relating to VTEC, these figures only give an estimate of the amount of research on these bacteria. Add to this the amount of unpublished information, or papers and posters presented at scientific meetings, which may not always get published in peer reviewed journals, it is evident that the scientific output of research relating to *E. coli* O157: H7 and other VTEC is substantial. Consequently, during this review it was sometimes difficult to keep pace with the progress and keep up-to-date with all of the

published research. However, every attempt was made to cover the important aspects of VTEC research, including new revelations, and to highlight any gaps in current knowledge. Moreover, this was achieved with the assistance of opinion leaders and researchers throughout the world who contributed to this review and whose contribution and opinions have been invaluable.

The ease with which scientific information can now be accessed, especially via the world wide web, has contributed to the wealth of information on VTEC, but especially VTEC O157: H7. The major down-side of this has been the sheer volume of information available and the time it now takes to sort through it, put the findings of research studies into context with what is already known and, more importantly, to make a judgement on the scientific integrity of some of this information. Whilst peer reviewed research has, at least, been scrutinised by other scientists, some information posted on the web may not have undergone the same checks. The information in this review was obtained largely from peer reviewed research but also from scientific and Government establishments and other reliable sources. The unpublished information included in this review was from respected and eminent scientists working on VTEC and some of this information has subsequently been published in peer reviewed journals or presented at conferences. Additional sources of information include Conference proceedings, books and published review articles. These also provide a valuable source of information on VTEC, which should also be consulted. Where relevant many of the published reviews on VTEC or related research have been cited in this review.

The following sections give a brief account of what is currently known about VTEC and some general conclusions from each section. For further information the reader should consult the individual sections of this review.

Section 2 Virulence determinants in VTEC

Our knowledge of these bacteria, their physiology, underlying genetics, the regulation and structure of the phage-borne Verocytotoxins (VT) and the regulation of virulence associated genes, has advanced at an impressive rate. This has been greatly aided by the development and widespread use of molecular methods and the publication of the complete genome sequences of a strain of *E. coli* K12 (Blattner *et al.* 1997) and two strains of *E. coli* O157: H7 (Hayashi *et al.* 2001; Perna *et al.* 2001).

As our understanding of *E. coli* O157: H7 and the underlying mechanisms of pathogenicity were revealed, it quickly became apparent that other *E. coli* had also acquired the VT encoding genes (*vtx/stx*). Consequently an entire pathotype of *E. coli*, which we now know as Verocytotoxin (Shiga toxin)-producing *E. coli* (VTEC/STEC), was born. The term enterohaemorrhagic *E. coli* (EHEC) is still used by some researchers, but this term has clinical connotations and is largely used to describe a sub-group of VTEC, including *E. coli* O157: H7, which is associated with strains causing distinct clinical manifestations, an ability to cause attaching/effacing lesions and carriage of a 60-MDa plasmid (Baylis *et al.* 2006). Furthermore, at the 6th International Symposium on Shiga Toxin (Verocytotoxin)-Producing *Escherichia coli* Infections (VTEC 2006), in Melbourne, Australia, a request to clarify or redefine this term was requested by scientists. It is therefore likely that the definition of EHEC will be discussed further in future and, if necessary, changed or dropped as a description for these bacteria.

Since the first description of the toxins and their cytotoxic activity against Vero cells by Konowalchuk (1977), our understanding of these toxins, and their structure, regulation and mode of action against eukaryotic cells, has advanced substantially. The differences between VT1 and VT2 are now more clearly understood, and it is appreciated that VT2 comprises a large family of closely related toxins or VT2 variants. However, the nomenclature of the toxins has changed over the years, especially with the introduction of newly described toxins or variant toxins. This has led to the introduction of new designations being used in the literature, which has led to much confusion. Attempts have been made by Scheutz *et al.* (2001) to clarify the designations used to describe the toxins, although it is likely that changes will occur in future. Although our understanding of the structure and regulation of these toxins has improved, gaps in knowledge remain in some areas. Control of virulence factors, including production of VT, has largely been determined from studies *in vitro*. How this relates to what happens *in vivo* is still unclear. There is also insufficient information on how some of the more recently described VT2 variants (e.g. VT2f, VT2g and VT2 NV206) compare with VT2 and VT2c and if VTEC carrying these are able to cause severe infection in man. Some revelations are also quite recent. For example, the route taken by VT from the intestines to the kidneys or other target organs was unknown for many years, until it was revealed by te Loo *et al.* (2000; 2001) that VT was possibly being transported in the blood by polymorphonuclear leukocytes. The pathway taken by the toxins and how they are processed and exert their action on eukaryotic cells has been described previously (Lingwood 1996; Sandvig and van Deurs 2002), although many of these studies have been conducted using VT1. However, some aspects of the toxin's pathway remain

unclear; for example, after the toxin molecule is transported retrogradely to the Golgi apparatus and endoplasmic reticulum (ER) of the host cell, the destination of the B fragment and A2 subunit is not yet fully understood (Baylis *et al.* 2006).

Host colonisation factors remain an important part of VTEC research, not only to improve our understanding of colonisation of animals but also their role in pathogenicity in animals and humans. Particular attention has focussed on the attaching and effacing (A/E) phenotype, first described in Enteropathogenic *E. coli* (EPEC) but now recognised in some strains of VTEC, notably *E. coli* O157: H7. The Locus of Enterocyte Effacement (LEE), and its organisation and regulation have been extensively studied. Consequently, much is now known about the LEE and the various proteins encoded by it. These include the type III protein secretion system, the adhesin intimin, which is encoded by the *eae* gene, and the translocated intimin receptor (Tir). Some gaps in research do, however, remain. Although a number of effector proteins associated with the LEE have been identified, it is not known if others, within or outside the LEE, exist and how these may exert their effect on virulence. Quorum sensing has been associated with the regulation of LEE operon promoters and regulation of virulence genes in *E. coli* O157: H7 (Sperandio *et al.* 2001; Anand and Griffiths 2003). The role of quorum sensing in virulence of VTEC, especially the part played by non-pathogenic bacteria in the intestine, remains unclear. However, not all VTEC possess the LEE, including some that have been associated with human infections. Consequently, attention has focussed on discovering alternative adhesins and colonisation factors. Several candidate colonisation factors have been reported (see Section 2.3.2 and Section 2.3.3), although other, yet to be discovered, colonisation factors may exist and how these are regulated and expressed would need to be determined. The immunological response to these factors in man and animals would also be of interest as this could assist with the development with potential vaccines in future. Furthermore, there is insufficient information on the ability of non-O157-LEE-negative VTEC to colonise ruminant or human intestines.

There are other factors in VTEC that are involved in pathogenicity, although the exact role they play in human disease remains unclear. Some of the genes encoding these putative virulence factors are carried on a 60MDa plasmid (pO157) in *E. coli* O157: H7 and other VTEC (Burland *et al.* 1998). One of the most notable plasmid-encoded pathogenicity-associated factors is enterohaemolysin; although there has been a suggested mechanism by which it exerts its effect on mammalian cells (Schmidt *et al.* 1996), the precise role of enterohaemolysin in VTEC infections is not fully understood. There are

many genes in VTEC that potentially encode other putative virulence factors, or which show similarity to known virulence factors in other pathogens (see Section 2.5). Many have not been studied so their function and precise role in disease have yet to be determined. How the regulation of these and other known virulence factors in VTEC in domestic animals are affected by diet and any stresses experienced by the animal, remain unanswered.

We now know of more than 400 recognised serotypes of *E. coli* that carry the *vtx* genes which are therefore classified as VTEC. However, the public health significance of many VTEC is not fully understood. Whilst much research has focussed on VTEC O157: H7, there is insufficient information on non-O157 VTEC and little is known about the exchange of *vtx* and other associated genes between other bacteria, including other members of the Enterobacteriaceae. In Germany, one reported outbreak of severe gastroenteritis followed by HUS and TTP in a nursery school, involved a strain of *Citrobacter freundii* carrying *vtx* genes (Tschape *et al.* 1995). There have also been reports of *vtx* genes being detected in a strain of *Hafnia alvei* isolated from fish and two strains of *Serratia liquefaciens* from minced meat (Linberg *et al.* 1998). Although there is evidence that horizontal gene transfer between closely related bacteria can occur, there is insufficient information on the extent of this and whether these bacteria will pose a potential food safety threat in future. Although many VTEC serotypes can be regularly isolated from particular animal species, many lack host specificity and indistinguishable strains can be isolated from a variety of animals as well as foods and the environment. Consequently VTEC display near ubiquitous distribution, although their role in human disease has yet to be fully understood.

Conclusions

- VTEC comprise a diverse group of *E. coli* that have evolved through the acquisition of additional genes (besides *vtx* genes which encode VT/Stx production).
- The clinical significance of many VTEC has yet to be determined.
- The arrangement and regulation of the Locus of Enterocyte Effacement (LEE) and the type III secretion system has been extensively studied in *E. coli* O157: H7 and some other VTEC serotypes. This has added to a greater understanding of the role of VTEC in colonisation of cattle and their role in human disease.
- The main mechanisms involved in the colonisation of cattle and other ruminants by *E. coli* O157 H7 have been identified and are now more clearly understood.

- Additional genes associated with colonisation and pathogenesis have not been fully elucidated (for example the identification and role of alternative genes or colonisation factors found in *eae*-negative VTEC associated with human disease).
- There is now a good understanding of the structure of the toxins and mode of action, yet the actual pathway used by VT (notably VT2) after passing through the host cell is still not fully understood.
- The role of quorum sensing and the contribution of other enteric bacteria in the exchange of virulence-associated genes and their potential role, if any, in disease caused by VTEC is not understood.

Section 3 VTEC Methodology

Not surprisingly, many of the developments in methods for VTEC have focussed on the detection and isolation of VTEC O157: H7. Strains of VTEC O157: H7 strains generally display two phenotypic characteristics that enable their differentiation from other *E. coli*, including other VTEC. These two features, the delayed/absence of rapid (within 24h) sorbitol fermentation and lack of β -glucuronidase (GUD) activity, have been exploited in methods for their detection/isolation (Baylis *et al.* 2001). The non-sorbitol fermenting (NSF) phenotype of VTEC O157: H7 has been used as a diagnostic marker to identify suspect *E. coli* O157 strains on Sorbitol MacConkey agar (SMAC). A modified version containing cefixime and potassium tellurite (CT-SMAC) provides additional selectivity for VTEC O157 since these organisms show resistance to tellurite compared with many other *E. coli*. CT-SMAC is the selective medium in national standard methods for the isolation of presumptive VTEC O157 from faeces and foods. The lack of GUD activity has been used as a supplementary test to confirm VTEC O157 colonies or to distinguish them from other *E. coli* or related bacteria on chromogenic media. Detection of VTEC O157: H7 has been aided by the introduction of immunological tests such as ELISA and lateral flow devices that enable detection of the O157 antigen in sample enrichments. More recently, molecular methods, which detect a number of O157-specific gene sequences, have developed and are now becoming more widely accepted. However, the cost of some tests can be a major factor that restricts their uptake for routine testing and in many clinical testing laboratories, conventional culture methods, typically involving direct plating onto selective diagnostic media, remain popular for routine screening.

The problem with methods developed to detect or isolate VTEC O157: H7 is their inability to detect or isolate SF, GUD-positive VTEC, such as strains of SF VTEC O157: H- or non-O157 VTEC. Unlike the majority of VTEC O157:

H7 strains, these VTEC strains have the same phenotype as general *E. coli*, so methods that rely on detection of phenotypic characteristics such as NSF and loss of GUD activity will not detect or identify them, especially if other *E. coli* or closely related bacteria are present. Consequently, methods developed to detect all VTEC or to confirm suspect VTEC colonies generally involve either immunological detection of VT, or molecular methods that detect the presence of the *vtx* genes alone or in combination with other genes associated with VTEC pathogenicity (e.g. *eae*).

There is general agreement that reliable primary isolation methods for pathogenic non-O157 VTEC are required that are applicable to samples taken from the environment, animals and all food types, for example media and incubation conditions that counteract any adverse effect of matrix physicochemical composition and competitor organisms on the recovery and growth of VTEC. Reliable isolation methods underpin all other work to characterise these organisms and facilitate our understanding of organism sources, survival capabilities, food chain transfer and controls and related VTEC public health issues.

Current gaps in knowledge relating to methods largely focus around the need for improved methods for the detection and isolation of non-O157 VTEC, although there is also a need to improve detection of SF VTEC O157. The introduction of IMS greatly improved detection of VTEC O157 from foods and animal samples (faeces) and this procedure has been included in an international standard method for the detection of *E. coli* O157 in foods and animal feeds (Anon 1999). The introduction of commercially available beads coated with antibodies specific to O111, O26, O145 and O103 antigens does enable better isolation of VTEC belonging to these serogroups. However, whilst these serogroups, together with O157, were once considered to be the most important five VTEC serogroups associated with human disease (World Health Organization Scientific Working Group 1999), using IMS for such a narrow range of serogroups does restrict testing to only a limited number of VTEC. Furthermore, cross-reactions between antibodies targeted against the O157 antigen with lipopolysaccharide structures in other bacteria have been reported (Baylis *et al.* 2001). It is not known whether cross-reactions can occur with antibodies targeted against other O antigens using immunological methods.

For general screening purposes, tests are often directed at detecting the presence of VT or *vtx* gene sequences, using ELISA or PCR-based methods, respectively. This approach provides a convenient screening method; however, the problem, which continues, is how to isolate and confirm which

VTEC are present in ELISA or PCR-positive samples. Currently, there are no satisfactory media for the isolation and identification of suspect VTEC, except for NSF VTEC O157. Consequently, confirmation of suspect VTEC isolates involves further screening of suspect colonies using molecular methods such as PCR or hybridisation assays. These are materials and labour intensive and it takes time to obtain colonies. It is essential, however, that colonies are isolated from foods and other samples to enable subsequent typing and characterisation of the VTEC present.

The reproducibility of typing methods is important, together with the accuracy and robustness of the method. For typing VTEC, phenotypic methods remain the most commonly used, although molecular methods are being introduced which either complement or have replaced some of the phenotypic ones. The most common phenotypic method used worldwide to type VTEC is serotyping. This is generally used together with toxin sub-typing and confirmation of associated virulence genes (e.g. *eae*) and occasionally, typing of intimin genes, to establish the serotype and virulence profile of the isolate. Phage typing has been used successfully to further type VTEC O157 strains, but this approach is limited to a small number of laboratories worldwide and is not suitable for non-O157 VTEC because of the number and diversity of these other serotypes. Molecular serotyping, which is quicker and is capable of typing all strains, has been developed, but uptake of this approach is still limited. For epidemiological investigations, pulsed field electrophoresis (PFGE) has been highly successful with VTEC and although it is currently the method of choice, there are limitations associated with this technique (see Section 3.8.2.2). Other typing techniques have been introduced, some with limited success, although multilocus variable number tandem repeat analysis (MLVA) is one technique that does appear to have a sensitivity equal to PFGE, and superior sensitivity.

The current gaps in knowledge remain the need for a standardised, internationally agreed typing scheme for VTEC, preferably using more rapid molecular methods such as sequence based typing methods, that can replace PFGE. Also, information is required to determine an accepted, validated sub-typing system for epidemiological typing of VTEC. If a particular strain is isolated from a food, patient or environment source, it is important that it can be definitely identified and linked to other isolates to establish the contamination route.

Understanding the association between human disease and the virulence profile and serotypes of VTEC strains responsible will be a major leap forward in understanding the public health significance of VTEC. This will be greatly

assisted by the introduction of better, more rapid typing methods. To screen large numbers of genes, microarrays are one way forward but they are currently being used by only a limited number of laboratories and not for routine diagnostic use. Compared with traditional typing and screening methods, microarrays require skilled operators and specialist equipment, which makes this approach too expensive to implement at present. As this and similar technologies become cheaper and simpler to operate, the uptake may increase.

Conclusions

- Methods for *E. coli* O157: H7 were quickly developed following outbreaks in the early 1980s. These methods are still being used today.
- The majority of methods for the detection and isolation of VTEC O157 rely on two key phenotypic characteristics shown by the majority of *E. coli* O157 strains:
 - Inability to rapidly ferment sorbitol (non-sorbitol fermenting NSF phenotype).
 - Lack of β -glucuronidase (GUD) activity.
- Future method development needs to address the detection and isolation of non-O157 VTEC, and to enable the detection/isolation of sorbitol fermenting (SF) and GUD-positive *E. coli* O157 strains.
- Detection of all VTEC strains relies on molecular approaches, especially the detection of *vtx/stx* sequences. Other gene targets e.g. *eae*, are important for the identification of VTEC which are more likely to be of clinical significance.
- The use of standardised methods (detection and typing) is essential for surveillance and survey purposes. Greater harmonisation of methods is needed in the future to aid epidemiology and to provide a greater understanding of VTEC prevalence in different countries.
- Despite improvements in detection methods, the isolation of VTEC remains difficult and labour-intensive. More diagnostic and specific methods for the isolation of suspect VTEC colonies is needed.

Section 4 Clinical aspects of VTEC infections

As a group, VTEC are responsible for a wide range of clinical manifestations in humans. These include asymptomatic carriage, uncomplicated or mild diarrhoea, haemorrhagic colitis (HC) and severe complications including haemolytic uraemic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP). The predominant serotype associated with HUS and severe

human disease is VTEC O157: H7, although more than 150 non-O157 serotypes have been associated with human disease (Bettelheim 2003). Severe disease in humans has been associated with age, with young children and the elderly at highest risk, and with carriage of certain virulence-associated genes, particularly *eae* and toxin type. The toxin types commonly associated with HC and HUS are VT2 and VT2c (see Section 3.9.2), whereas VT1 is less frequently associated with disease in humans.

Despite our better understanding of disease caused by VTEC, especially VTEC O157: H7, there are many aspects of human disease that are not fully understood. Routine serodiagnosis has been used for several years yet the kinetics of antibody production by patients infected with VTEC O157: H7 is still poorly understood. Even less is known about infections caused by non-O157 VTEC, and asymptomatic carriage of VTEC in humans and the infective dose of these bacteria is not known or is not completely understood. To understand these aspects of VTEC infection better, more information is needed on cases and their close contacts during outbreaks. Detailed follow-up reports on cases over a twelve month period would help to provide this information.

It is apparent that there is a need for internationally standardised diagnostic criteria for VTEC infection. Treatment of patients with VTEC infections, especially the use of antibiotic treatment, remains controversial. Scientific evidence would suggest that certain antibiotics may actually induce toxin production by VTEC and therefore increase the risk of patients developing HUS and other complications. This risk is not known or completely understood by some medical practitioners and antibiotic treatment can often be administered before a patient's infection has been diagnosed. Anti-diarrhoeal and anti-motility agents may also precipitate HUS/TTP and should also be avoided for the treatment of patients with acute gastrointestinal infection by VTEC. Current management of patients is therefore supportive and aimed at the management of complications such as fluid and electrolyte imbalance. Although plasmapheresis (therapeutic plasma exchange) has been used as a therapeutic tool for the treatment of HUS in the past, this approach may only be effective when administered before onset of acute renal failure (Dundas and Todd 2000). Dundas and Todd (2000) have further commented that this approach is expensive and its role in the treatment of adults with HUS and TTP needs to be determined by randomised controlled trial or by establishing an international register of *E. coli* O157 induced HUS and TTP. Use of plasmapheresis therefore remains controversial.

More definitive guidance on treatment of VTEC infections, supported by scientific evidence, is needed. There also needs to be a better understanding of how general practitioners and paediatricians approach the management of bloody diarrhoea, especially in children. Much of the data on VTEC O157 infection has been derived from studies with children because these are at greatest risk and are the group most commonly associated with severe disease, including HC and HUS. Consequently there is less information available on the clinical course and outcome of VTEC infections in adult humans. A national register of VTEC cases, which already exists in Scotland and which should be considered in other countries, including England and Wales, would help to address this gap in knowledge.

The role of non-O157 VTEC in human diarrhoeal disease is not fully appreciated. In some countries, non-O157 VTEC are either not looked for in clinical specimens, or the methods used by many laboratories are specifically designed to isolate NSF VTEC O157, and inappropriate for the isolation of a wide range of VTEC serotypes. This aspect of VTEC research should improve with the introduction of better methods for VTEC, although the cost and time needed to screen for VTEC will be important factors that will influence this. Moreover, the clinical significance of many non-O157 VTEC remains unknown, especially those found in animals, foods and the environment. With a better understanding of these bacteria and their role in human disease, it will be possible to administer more appropriate treatments and to implement more effective interventions during outbreaks. There is a lack of evidence about the effectiveness of alternative therapies, including the potential role of probiotics in disease management. Attempts have been made to develop human vaccines against VTEC O157: H7 but these have proved ineffective.

Conclusions

- VTEC are responsible for a range of clinical manifestations in man ranging from mild diarrhoea through to haemorrhagic colitis and life threatening haemolytic uraemic syndrome (HUS).
- HUS remains one of the most serious manifestations of infection by VTEC.
- The concept of seropathotype (association between serotype and virulence gene profile, e.g. toxin subtype, presence of *eae* and disease outcome) provides a useful starting point for determining disease potential. The development of this approach should assist with the identification of VTEC likely to cause human disease.

- There is a need for a better understanding of how paediatricians and general practitioners manage VTEC infections, especially bloody diarrhoea in children.
- Treatment of VTEC infection should be considered carefully and the use of certain antibiotics should be avoided. Management of patients is currently supportive.
- The development of a human vaccine has not been successful although research into this continues.
- There is insufficient knowledge of VTEC serotypes associated with asymptomatic carriage.

Section 5 Epidemiology of VTEC in humans

The link between VTEC and human disease became apparent in 1982, following outbreaks of haemorrhagic colitis in the USA that were linked to VTEC O157: H7 and consumption of hamburgers (Riley *et al.* 1983). However, it is likely that VTEC were around before this time and possibly responsible for outbreaks of infection in the USA in the 1950's (Bettelheim 2000). Since 1982 there have been outbreaks of human infection associated with food, water and environmental contamination. Animals, especially ruminants (cattle and sheep), are important reservoirs for VTEC O157: H7, and contaminated foods (e.g. meat, milk and dairy products) have caused outbreaks and cases of sporadic infection. Farm waste, manure and animal faeces have been responsible for outbreaks of infection, either by contamination of fresh produce or via human direct contact with contaminated animals, land or water.

Despite the 'domination' of VTEC O157: H7 in the UK and USA, it is important to understand why non-O157 (such as O26) 'dominate' in other European countries, Japan, Australia, etc. Possible factors could be agricultural and animal husbandry practices, consumer-eating habits, food processing systems and, perhaps, even geographical influences. A better understanding of VTEC in relation to these factors is needed as this could help us to understand, in turn, why some pathogenic non-O157 VTEC appear to be more prevalent in some countries and not others, and also, how control of these factors can influence the emergence of some VTEC serotypes which subsequently become of greater public health importance in that country.

Differences in VTEC surveillance between countries, including differences in diagnostic and clinical practices, isolation and typing methods and reporting of VTEC infections, all influence the amount and quality of information available. There are several aspects of VTEC surveillance that currently restrict

comparison of data and prevent better understanding of the global situation concerning VTEC. These include:

- Lack of surveillance data on non-O157 VTEC
- Lack of complete information on HUS
- Limited information on non-O157 VTEC causing HUS
- Limited information on VTEC seasonality in animals and humans
- Limited information on age-related prevalence of VTEC infections
- Limited information on asymptomatic carriage

As a result, establishing which VTEC serotypes are most commonly associated with disease in different countries and establishing trends or geographical differences is often difficult. Improved global surveillance systems for VTEC infections are needed.

In the UK, where VTEC O157: H7 is the predominant serotype, it could be assumed that the prevalence of this serotype is attributed to the methods used which screen for NSF O157 and the limited amount of testing for non-O157 VTEC in the UK. In contrast, other European countries, such as Germany, often screen for VTEC initially, usually by testing for VT or vtx and then focus their efforts on isolating NSF VTEC O157. However, since the recommendation of the *E. coli* O157 Task Force Report in June 2001 (Anon. 2001) that faecal samples from patients in Scotland should also be tested for non-O157 VTEC by the Scottish *E. coli* O157 Reference Laboratory (SERL), very few non-O157 VTEC have been reported. Similar findings have also been obtained by the Health Protection Agency in England and Wales, thus supporting the evidence that VTEC O157: H7 is the predominant serotype in the UK. Scotland has the highest recorded rate of VTEC O157 infection in the UK yet the reasons for this have yet to be explained. Possible reasons include greater exposure of people in parts of Scotland to environmental contamination, drinking from untreated water supplies by rural communities in some areas and differences in the prevalence, concentration and shedding of VTEC O157 in animals in this country. Studies investigating prevalence of VTEC O157 in Scottish livestock have focussed on determining the numbers of these bacteria being shed by individual animals. Equivalent data for livestock from other countries, including England & Wales, could help to reveal if differences existed between the prevalence and the concentration of VTEC O157 being excreted by livestock in these countries. Whether these differences could account for the disparity in infection rates between Scotland and other counties remains unclear. However, it is likely that the answer is not a simple one and that there are multiple factors that contribute to Scotland having such a high rate of VTEC O157 infection.

It is now apparent that VTEC are widely disseminated in nature and can be isolated from a variety of animals and environmental habitats, although our knowledge of VTEC in animals, other than cattle and sheep, is still limited. Humans can therefore become infected by direct contact with contaminated animals or their faeces, eating contaminated foods and from ingestion of contaminated water. Ruminants, such as cattle and sheep, were among the first animals to be identified as reservoirs for VTEC O157: H7 and there have been many outbreaks and cases of infection linked to foods, especially meat derived from these animals. Unpasteurised milk and dairy products feature prominently in foodborne outbreaks; in the USA, unpasteurised, unfermented apple cider has been implicated in outbreaks of VTEC O157: H7 infection. More recently, fresh produce, particularly sprouted seeds and lettuce, have caused outbreaks in Japan, the USA and to a lesser extent other countries. Waterborne VTEC infections have been attributed to drinking untreated water, treatment failures associated with municipal water supplies and ingesting water during swimming in contaminated recreational water or swimming pools. In outbreaks, person-to-person transmission is an important contributing factor in the spread of infection, although shedding of VTEC by asymptomatic carriers may also be an important risk factor which is not completely understood.

Conclusions

- The majority of VTEC infections are sporadic.
- Person-to-person transmission (secondary spread) of VTEC represents a real risk among family members and the wider community.
- The importance of non-O157 VTEC in human infection is not fully understood.
- Geographical (international and national) differences in the incidence of VTEC prevalence and infection have not been fully explained.
- In the past a lack of standardisation of test methods and data collection, and the lack of harmonisation between national surveillance networks have prevented comparison of information between countries. In Europe this deficiency in the surveillance network has been recognised and is being addressed.

Section 6 Reservoirs for VTEC and the food chain

The mechanisms of infection and routes of transmission for VTEC O157 from cattle to man is well understood. However, there is less known about transmission of VTEC from the environment to animals and humans, and transmission of VTEC between different animals, the exception being transfer

of VTEC O157 between cattle prior to slaughter, which has been extensively studied. Although information on non-O157 VTEC in cattle and sheep, including associated prevalence data, is plentiful for sheep and cattle in some countries, notably Spain, Germany and other European countries, USA and Australia, this information is not available for other countries. There is also limited information on the ecology of non-O157 VTEC in the environment, including which serotypes are most prevalent, the virulence genes carried by these bacteria and any possible host associations with wild animals that could act as reservoirs for the transmission of VTEC to domestic animals or man. As well as transmission of VTEC, there is limited information currently on the ecology of *vtx* carrying phages in the environment and their role in horizontal transmission and the dissemination of *vtx* genes among bacteria in the environment.

Domestic animals, especially ruminants such as cattle and sheep, have long been associated with carriage and shedding of VTEC O157: H7. "Super shedders" or high shedding animals (cattle and sheep) have been shown to excrete *E. coli* O157 at levels $> 10^3$ cfu/g in their faeces. These animals pose a significant contamination risk to other animals on the farm as well as to the environment. They also represent a serious threat to the food chain if presented for slaughter. Although studies that have determined the concentration of VTEC O157 being excreted by individual animals have been

As researchers have widened their search for VTEC in other animal species, it has become apparent that these bacteria, especially non-O157 VTEC, are widely distributed and can be isolated from many different species of animals, including mammals, birds and insects. Contaminated faeces, especially those from farm animals, or manure and slurry from farms can then introduce VTEC into the environment, contaminating farm land, streams and rivers, and ultimately introduce them into the food chain via contaminated fresh produce. Foods such as meats, milk and dairy products and products derived from these can all become contaminated by VTEC. Data now exists on the prevalence of VTEC O157 in cattle and sheep and surveys have established the prevalence of VTEC O157 in raw meats, especially beef. Differences in sampling methods and detection/isolation techniques, in the past, have prevented direct comparisons between some studies from being made. Improved standardisation of methods, the introduction of IMS and better isolation procedures have improved this situation.

There is limited information on the prevalence of VTEC O157 in many foods, with the exception of those previously implicated in outbreaks. These include raw meats, especially beef and lamb, raw meat products, raw milk and dairy

products made from unpasteurised milk. For these types of foods, data is available, although this is largely restricted to developed countries in the Western World, in particular the UK and other European countries, the USA, and Canada. There remains limited information on VTEC in foods, including raw meats, from countries in the East, Middle East and Far East. Data on non-O157 VTEC in foods is becoming more widely available, although this is still limited to those countries that have the methods to screen for VTEC, besides NSF O157, isolate them and type them. Our current lack of understanding of non-O157 VTEC and their clinical significance prevents us from appreciating the potential risks they may pose when they enter the food chain. Even less is known about their routes of transmission, prevalence and persistence in many foods. Research in this area would lead to better, more appropriate intervention systems/strategies for controlling the spread of VTEC in the food chain.

Conclusions

- Cattle and sheep remain primary reservoirs for VTEC, especially VTEC O157: H7. Consequently foods or products derived from these animals pose the greatest risk of VTEC contamination.
- "Super shedders" or high shedding animals (cattle and sheep) have been shown to excrete *E. coli* O157 at levels $>10^4$ CFU/g in their faeces. These animals are an important
- Although meat and meat products (especially burgers) have long been associated with *E. coli* O157: H7 outbreaks, the incidence of this pathogen in raw meats is generally low, although occasionally there have been reports of higher levels in raw beef.
- The incidence of non-O157 VTEC in raw meats is higher than for VTEC O157: H7 although many of these strains are *eae*-negative and the clinical significance of these VTEC is unknown.
- The significance of VTEC in food, especially raw meats, and the potential food safety risk from it is not fully understood and requires careful consideration in the future.
- Despite the common association that exists between VTEC O157: H7 and raw beef, VTEC serotypes can be present in non-meat foods, although these usually have a direct (e.g. milk) or indirect (e.g. environmental contamination of produce) link to the primary reservoirs.
- There is a lack of data on the prevalence of VTEC (especially non-O157 VTEC) in foods from developing countries and other countries which export produce and other foods.

- The role of other animals (wild and domestic) as vectors for the transmission of VTEC in the environment and their entry into the food chain is not fully understood.
- Environmental exposure (direct and indirect contact with contaminated animals, farmland, water etc) represents a significant risk factor for VTEC infection.
- There are multiple transmission routes by which VTEC can get into the food chain or infect humans directly.

Section 7 Prevention and control of VTEC contamination

It has become evident from food outbreaks that VTEC O157: H7, along with other VTEC can enter the food chain. Carriage and shedding of VTEC O157: H7 by cattle has been established and it has become clear that these bacteria can enter the food chain during primary production and that contaminated raw meat is an important vehicle in outbreaks of infection. As the number of VTEC outbreaks and the types of foods involved has increased, so too has our understanding of the potential routes of transmission and associated risk factors. No longer are outbreaks just associated with products derived directly from animals, such as meats and meat products, but fresh produce and sprouted seeds have featured in several recent outbreaks of VTEC O157: H7 infection.

The 'farm-to-fork' policy of food safety adopted by the UK Food Standards Agency and other Governments worldwide has been instrumental in reducing the number of VTEC related outbreaks and for reducing or eliminating VTEC in primary food production. In the UK and other European countries, general policies implemented to prevent foodborne illness from a range of bacterial pathogens such as *Salmonella*, *Campylobacter* and *Listeria monocytogenes* have been equally effective against VTEC. Preventing contamination in the first place has been the primary objective in the UK and the introduction of the clean livestock policy is one example of a measure introduced to reduce contamination entering the slaughterhouses and the food chain. In contrast, the USA has introduced policies to directly combat VTEC O157: H7 and although some appear to be working, large outbreaks of VTEC O157: H7 infections have occurred recently. However, differences in the way that food is produced and distributed in the US may also account for this. Contaminated fresh produce is of particular concern in the US where the number of outbreaks caused by these foods has been increasing. The adoption of Hazard Analysis and Critical Control Point (HACCP) systems has played an important part in ensuring food safety in the food industry and the

principles of HACCP have also been adopted further up the food chain in primary production and in the catering sector.

Various physical and chemical treatments have been developed to remove or eliminate VTEC at various stages of the food chain. Some of these treatments have been shown to be effective against VTEC O157: H7 although some are not permitted for use in certain countries and others have not been proven to be safe to use on foods intended for human consumption, so these are not currently permitted. There has been considerable research on preventing VTEC colonisation of cattle and other animals entering the food chain. Attention has also focussed on introducing intervention measures to prevent transmission of VTEC between animals on the farm. This has included treating drinking water and decontaminating water troughs but also investigating housing and animal husbandry practices. Pre-slaughter interventions for cattle have received much attention, especially in the USA where the USDA has conducted research into the effects of various treatments. Among the interventions investigated for their ability to reduce or eliminate VTEC in cattle prior to slaughter are finishing diets, competitive exclusion and probiotics, dietary supplements, immunisation and bacteriophages (Callaway *et al.* 2004). Even though some of these treatments have proven effective against VTEC O157: H7, there is no evidence that they will be equally as effective against non-O157 VTEC. With some treatments, such as use of finishing diets, there remains concern over whether the treatment could inadvertently encourage colonisation or survival of VTEC. Use of antibiotics to remove VTEC from cattle and other animals remains controversial, although some would argue that this approach warrants further investigation. Others would prefer to avoid the use of antibiotics in animals to reduce the potential risk of antibiotic resistant strains of bacteria emerging and entering the food chain.

Cattle hides have been shown to be important sources of VTEC and subsequent introduction of VTEC contamination into the abattoir where the carcasses and the surrounding environment become contaminated. Ensuring that cattle are clean prior to slaughter has been adopted in the UK as a measure to reduce the risk of VTEC contamination. In the USA, spraying cattle hides with cetylpyridium chloride (CPC) has been investigated but there has been limited uptake and for animal welfare reasons this approach is only suitable for stunned animals prior to hide removal. Various treatments have been introduced for carcass decontamination, including washing and trimming, hot water treatment, steam pasteurisation and use of organic acids and chemical treatments. Some of these treatments appear to be effective, although many of the chemical treatments developed and evaluated in the

USA are not permitted in Europe or other countries outside the US. There is currently insufficient understanding of the interactions between VTEC and the carcass or meat surfaces and whether pili or other mechanisms of attachment prevent their removal by washing or other physical treatments. If VTEC are present in raw meat and meat products, adequate cooking and avoiding cross-contamination during handling and preparation of food remain the most effective control measures. The inhibitory effect of competitor organisms against VTEC in raw meat and other foods has received attention but there is insufficient evidence that this approach can be used to control or eliminate VTEC.

The risk from fresh produce contaminated with VTEC has received much attention in recent years. This is largely because of the rise in the number of VTEC O157: H7 outbreaks associated with fresh produce and because of the obvious risk presented to produce by contaminated irrigation water, manure and contaminated soil. There are a number of problems associated with fresh produce, not least the difficulty in decontaminating fruits and vegetables and removal of bacteria from the leaves and stems of plants. Washing has limited effectiveness and some chemical treatments, although effective, are either not permitted in the UK and other countries, or they have an adverse effect on the organoleptic or visual qualities of the product. There still needs to be a better understanding of the intimate attachment of VTEC to the surface of raw fruits and vegetables. Internalisation of VTEC in plant tissues, either as a result of growing plants from contaminated seeds, or by the phenomenon termed aspiration, has been investigated but more evidence is needed to fully understand the extent of this problem.

Traditional hygiene measures such as disinfection and good manufacturing practice, underpinned by HACCP or a similar risk based management system, remain the most common approaches used by the food industry to ensure food safety. During food manufacture, a variety of conventional and novel treatments have proven effective against VTEC O157 H: 7 and other vegetative pathogens. Heat treatments have been extensively studied, especially for their effectiveness in destroying VTEC O157: H7 in meat and meat products and in unpasteurised, unfermented apple cider, which is a popular drink in the USA. Given the undesirable effects of heat on the sensory quality of some foods, there has been considerable research done on non-thermal treatments against VTEC O157: H7. Among the treatments investigated are high pressure, ultrasound, UV and gamma irradiation, acids and chemical preservatives, natural antimicrobial compounds and pulsed electric field. These have shown varying degrees of effectiveness, although

there is insufficient evidence of their effectiveness against a wide range of VTEC and most require further evaluations to determine their true potential.

Besides foods, water and environmental contamination have also become increasingly important. Whether private water supplies in rural communities are responsible for VTEC infections has yet to be fully determined. Treating manure to destroy pathogens, including VTEC, has been investigated but there is insufficient data to demonstrate the full effectiveness of these treatments. Environmental contamination and contaminated farm animals have been highlighted as potential risk factors for acquiring VTEC infections. This has been demonstrated by outbreaks associated with farm parks where children have come into direct contact with contaminated animals. There have also been documented cases of humans becoming infected by VTEC after camping on, or visiting, land contaminated with sheep or cattle faeces. Controls have been introduced in many countries to reduce the risk of VTEC transmission from contaminated animals and land to humans. This has largely been by education and by ensuring that people follow basic hygiene procedures, such as washing hands after touching animals and before eating. To prevent contamination of land and crops, there are various guidelines and codes of practice which cover the management of animals on pasture and the application of manure, sewage sludge and water on agricultural land.

Predictive modelling has become a valuable tool used by the food industry to determine whether a particular pathogen will survive or grow under the conditions found in a food product or environment. There are very few models for non-O157 VTEC, although these bacteria may be expected to behave in a similar way to *E. coli* O157: H7 or general *E. coli*, for which there are models available. One of the main limitations of current predictive models is their inability to predict survival/growth at levels close to the minimum growth requirements of the organism. This is partly because it has been difficult to accurately measure growth under these conditions. Lastly, microbiological risk assessment (MRA) has played an important role in the development of HACCP plans and hazard identification, although there still needs to be a better linkage between MRA and HACCP. In the future, the use of different mathematical approaches could bring about improved models for use in MRA. Testing mitigation strategies in risk assessments also warrants more attention in future.

Conclusions

- The primary aim of some countries, including the UK, has not been to develop treatments to remove VTEC contamination but to prevent contamination occurring in the first place.
- Controls and intervention strategies targeted against a range of vegetative pathogens, e.g. *Salmonella*, should also be effective against VTEC.
- Good agriculture practices at every stage of fresh produce production is instrumental in preventing the risk of VTEC contamination.
- Post harvest treatments for produce are limited, so avoiding contamination in the first place should be the primary objective.
- Preventing colonisation of cattle has been extensively studied and there are several strategies and interventions that have been investigated. Examples include:
 - animal husbandry practices, e.g. housing and grazing of animals
 - modifications to diet
 - competitive exclusion
 - vaccination of cattle
 - bacteriophage treatment of hides
- The vaccination of cattle against *E. coli* O157, which has been progressed in the US, offers a potential way of successfully preventing or reducing colonisation.
- The hides of cattle have been highlighted as a significant source of VTEC contamination, especially during their removal in the slaughterhouse.
- Ensuring that animals entering the slaughterhouse are clean (especially the removal of faecal material) has been an important control implemented in the UK (clean livestock policy) and other countries to reduce the level of contamination.
- Preventing VTEC infection by foods relies on the same principles applied to other foodborne pathogens, namely the proper cooking and handling of foods potentially contaminated with VTEC (e.g. raw meats).
- The adoption of HACCP principles by industry and more recently by caterers will contribute to safer food and reduce the risk from VTEC.
- Better education of everyone preparing or handling foods (caterers as well as the general public) is needed to reduce the burden of foodborne disease and the risk of VTEC infection.

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