

**UK Publicly Funded Research Relating to  
Verocytotoxin-producing *Escherichia coli*  
(VTEC): Update, September 2004**

**Research covered from 1999 to 2003**

**Report to the Microbiological Safety of Food  
Funders Group**

**[October 2004]**

## **UK Publicly Funded Research Relating to Verocytotoxin-producing *Escherichia coli* (VTEC): Update, October 2004**

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#### **SUMMARY**

The report *UK Publicly Funded Research Relating to Verocytotoxin-producing Escherichia coli (VTEC)* has been updated for the Microbiological Safety of Food Funders Group (MSFFG). The new report covers research funded by the members of the MSFFG in a total of 102 projects over the period 1999 to 2003. The predominant research areas, have been the pathogenicity and epidemiology of VTEC and the reduction and elimination of the organism from cattle and the food supply chain.

Whilst VTEC infections are rare, they represent a serious public health issue as the organisms can have a very low infectious dose and the consequent illness is both serious and can lead to death. There are also potential complications, in particular haemorrhagic colitis and haemolytic uraemic syndrome (HUS). These public health issues underpin the need for research into the organism, as does the awareness that although infection is generally food-borne, there are increasing numbers of cases now attributable to environmental sources.

#### Significant advances achieved through research

During the period of the report it has been recognised within the UK and internationally (Duffy, Garvey and Sheridan (2002)) that a vital control point in order to reduce the numbers of VTEC entering the human food chain is the management of animal waste (farm and abattoir). This understanding has been based on studies of the pathogenicity of the organism and its epidemiology. It is possible that this understanding, and the consequent changes, are contributing to the reduction of the numbers of reported VTEC infections in the UK in recent years.

There have also been very substantial advances in the understanding of the infection and colonisation of cattle by VTEC. Good management of funding resources in this area will undoubtedly continue to lead to fruitful research.

#### Outstanding issues

Despite these major advances, there remain a number of areas of research where there is a need for further work. These include:

- A better understanding of the epidemiology of the transfer of VTEC from animals and the environment to man. For example, research to determine whether the types of VTEC which are found in farm animals are the same as those found in human disease. Such research would need to ensure that all routes of human infection are addressed, including farm and wild animals (eg

deer, rabbits, etc), the environment (water sources, fields etc), food products and human-to-human transmission;

- The agreement, internationally, of objective, standardised techniques and systems for typing the different strains of *E. coli*, and the use of those methods in the quantification of the VTEC and other strains of *E. coli* in different environments;
- A greater understanding of the pathogen:host interaction between VTEC (and other forms of pathogenic *E. coli*) and man;
- There is only limited research to determine the risk of transfer of the virulence determinants from VTEC to other organisms.

The research covered in this report falls into two parts: research to understand the fundamental biology of VTEC and, generally separately, research to address the public health issues, in particular the reduction and elimination of VTEC from the farm and food chain. Greater engagement between these different aspects of VTEC science could be beneficial to all aspects of the research.

## LAY SUMMARY

*Escherichia coli*, generally known as *E. coli*, is a common bacterium, which is found in the digestive tract of humans and animals. Most strains of *E. coli* are harmless, but those that produce a particular toxin (called verocytotoxin-producing *E. coli*, or VTEC) can cause severe illness. In the UK, the most frequently observed type of VTEC in man is *E. coli* O157. *E. coli* O157 is transmitted to people most commonly through undercooked minced beef and milk that is raw, inadequately pasteurised or contaminated after pasteurisation. It is also possible to become infected by direct contact with infected animals or people, and by contact with land contaminated with animal faeces. General symptoms include bloody diarrhoea and abdominal cramps. The illness can also have very serious complications, including kidney failure, severe anaemia and neurological problems. On rare occasions, *E. coli* O157 infections can lead to death.

Although uncommon, a small number of serious outbreaks of illness caused by VTEC have made it a public health issue. This has prompted research to understand how the bacterium causes illness, and how people become infected with it. Research covering these areas, as well as issues such as how to know that an infection is caused by *E. coli* O157, is described in this report.

The numbers of reported *E. coli* O157 infections in the UK are falling, possibly in part because of improved farm and food handling practices.

## TABLE OF CONTENTS

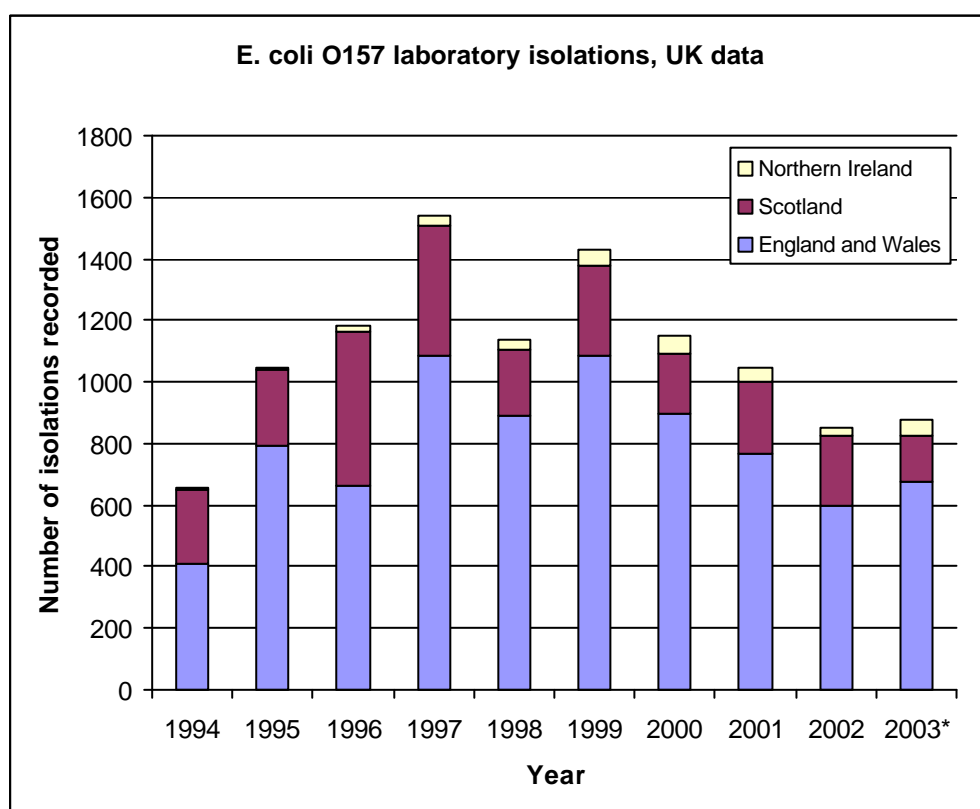
SUMMARY.....	2
LAY SUMMARY.....	4
TABLE OF CONTENTS.....	5
INTRODUCTION .....	6
1. BACKGROUND.....	6
2. DEFINITIONS.....	8
3. METHODS .....	9
4. RESEARCH FUNDED BY OTHER FUNDING BODIES .....	10
MSFFG FUNDED PATHOGENIC <i>ESCHERICHIA COLI</i> RESEARCH 1999 TO THE PRESENT.....	13
5. DETECTION, DIFFERENTIATION AND DIAGNOSIS.....	13
6. MICROBIAL PHYSIOLOGY AND GENETICS .....	17
7. PATHOGENICITY.....	20
8. EPIDEMIOLOGY.....	25
9. REDUCTION AND ELIMINATION .....	30
10. MICROBIAL ANTIBIOTIC RESISTANCE.....	34
11. SURVEILLANCE.....	34
12. RISK ASSESSMENT .....	34
13. OTHER.....	37
14. CONCLUSIONS .....	38
REFERENCES .....	40
APPENDIX 1: GLOSSARY .....	41
APPENDIX 2: PROJECTS FROM THE MSFFG DATABASE USED IN THIS REPORT.....	44

## INTRODUCTION

### 1. BACKGROUND

- 1.1 *Escherichia coli* is a commonly occurring bacterium found in the digestive tract of man and animals. Most strains of *E. coli* are harmless, but those that produce verocytotoxin (verocytotoxin-producing *E. coli* or VTEC) can cause severe illness. In the UK, the most common type found in man is *E. coli* O157. The first incidence of *Escherichia coli* O157:H7 as the cause of gastrointestinal illness was reported in 1982 (Riley *et al*, (1983)). UK reports of illness due to the organism have increased since then, reaching a peak between 1997 and 1999. There were 851 laboratory confirmed cases of VTEC in the UK in 2002 and 876 in 2003 (see Figure 1 below). The numbers of food-borne associated outbreaks of *E. coli* O157 were six in 2000 and one per year for 2001 to 2003.

**Figure 1: Laboratory confirmed cases of O157 VTEC in the UK 1994 to 2003<sup>1</sup>**



\* 2003 data are provisional

<sup>1</sup> [http://www.hpa.org.uk/infections/topics\\_az/ecoli/O157/facts.htm#trends](http://www.hpa.org.uk/infections/topics_az/ecoli/O157/facts.htm#trends),  
[http://www.cdscni.org.uk/surveillance/Gastro/Escherichia\\_coli\\_O\\_157.htm](http://www.cdscni.org.uk/surveillance/Gastro/Escherichia_coli_O_157.htm),  
<http://www.show.scot.nhs.uk/scieh/>

- 1.2 Concern relating to the apparent emergence of *E. coli* O157 as a new human intestinal pathogen prompted a report by the Advisory Committee on the Microbiological Safety of Food (ACMSF) on verocytotoxin-producing *E. coli* (*Report on Verocytotoxin-Producing Escherichia coli* (1995)<sup>2</sup>). In 1996 an outbreak of food poisoning in North Lanarkshire, Scotland, led to 17 deaths and illness for a further 496 people. The situation was regarded as being of sufficient severity that it led not only to the Pennington Group report<sup>3</sup> but to an increase in the general attention paid to the pathology and biology of the organism (eg Task Force on *E. coli* O157: Final report (2001)<sup>4</sup>, Report of the Chief Medical Officer, (2002)<sup>5</sup>). Much of the effort relating to *E. coli* O157 has focused on public health issues, and approaches to reducing and eliminating the organism from the food chain. However, there has also been an active effort in research related to the biology of the organism, results of which should inform the development of government and public policy.
- 1.3 VTEC O157 is the most commonly reported serogroup isolated from man in the UK, USA and other developed countries (Nataro and Kaper (1998)). Non-O157 serogroups of VTEC have been associated with outbreaks of disease elsewhere, including in some European countries. As well as O157, other serogroups of human importance are O145, O111, O26 and O103 (WHO Scientific Working Group(1999)<sup>6</sup>).
- 1.4 In October 1999 the Microbiological Safety of Food Funders Group (MSFFG) published a report entitled: *UK Publicly-funded research relating to verocytotoxin-producing Escherichia coli* (VTEC) (hereafter referred to as 'the 1999 MSFFG VTEC report'<sup>7</sup>). This report provided an overview of research related to verocytotoxin-producing *E. coli* O157 (VTEC O157) being undertaken in the UK and funded by the members of the MSFFG. The report summarised progress in UK publicly funded VTEC research for the period 1990 to 1999 within a number of research areas and in the context of issues raised in the UK and overseas relating to the emergence of *E. coli* O157 as a human pathogen. In addition, an assessment was made of those areas where further research might be needed.
- 1.5 Four years later, it is appropriate for the 1999 MSFFG VTEC Report to be updated. This will give an overview of the progress which has been made in understanding of VTEC and associated *E. coli* research, in particular through UK publicly funded work. In addition, this updated report will contribute to a Food Standards Agency (FSA) review of FSA- funded

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<sup>2</sup> <http://www.food.gov.uk/science/research/researchinfo/foodborneillness/microfundervtec>

<sup>3</sup> <http://www.scotland.gov.uk/deleted/library/documents-w4/pgr-00.htm>

<sup>4</sup> <http://www.foodstandards.gov.uk/multimedia/pdfs/ecolitaskfinreport>

<sup>5</sup> <http://www.doh.gov.uk/cmo/annualreport2002/progress.htm>

<sup>6</sup> <http://www.who.int/emc-documents/zoonoses/whocsrph988c.html>

<sup>7</sup> [http://www.foodstandards.gov.uk/science/research2/research\\_archive/pubfund/microbiosafe/vtec.html](http://www.foodstandards.gov.uk/science/research2/research_archive/pubfund/microbiosafe/vtec.html)

VTEC research, to be undertaken in early 2005.

- 1.6 The principal areas of VTEC research where there has been substantial effort are in the exploration of analytical methods for detecting various forms of *E. coli* (section 5), research into pathogenicity (section 7) and epidemiology (section 8) as well as mechanisms for the reduction and elimination of the bacterium from the food chain (section 9). In addition, there has been work in areas such as microbial physiology and genetics (section 6) and risk assessment (section 12). Each section of this report addresses the progress that has been made, revisits the aspects of research that were identified as requiring more effort in the 1999 MSFFG VTEC Report and then considers those areas where further research might be needed.

## 2. DEFINITIONS

- 2.1 In the 1999 MSFFG VTEC Report, only research involving serotypes of *E. coli* which produced verocytotoxin was included. In practice, this meant that the report covered research with *E. coli* O157 as this was the most frequent serotype isolated in the UK. However, with increasing research effort, and rising awareness of the significance of other serotypes of VTEC to the burden of human disease, it has been decided that this report will address research involving any verocytotoxin-producing *E. coli* (VTEC) that are pathogenic to man and of importance in food safety. The serotypes include for example *E. coli* O26, O111, O103, and O145. In practice, it remains the case that much of the research in the UK with *E. coli* uses the wild-type organism *E. coli* K12, or *E. coli* O157.
- 2.2 Most *E. coli* are non-pathogenic and are part of the normal bowel flora in humans and animals. *E. coli* associated with diarrhoeal disease are referred to collectively as enterovirulent *E. coli*. Seven groups have been defined based on various virulence factors including toxin production and adhesion (Nataro and Kaper (1998)). The seven pathogenic groups are:
- Attaching and effacing *E. coli* (AEEC)
  - Diffusely adherent *E. coli* (DAEC)
  - Enteroaggregative *E. coli* (EaggEC)
  - Enteroinvasive *E. coli* (EIEC)
  - Enteropathogenic *E. coli* (EPEC)
  - Enterotoxigenic *E. coli* (ETEC),
  - Verocytotoxin-producing *E. coli* (VTEC).
- 2.3 *E. coli* types are classified in the laboratory by the structure of the O and H antigens found on the bacterial cell surface. Using this approach, there are more than 170 O-serogroups which can in turn be further subdivided using the H antigen into serotypes. Hence the majority of *E. coli* associated with disease in man are *E. coli* serogroup O157, and within this, the most commonly observed serotype is *E. coli* O157:H7.

- 2.4 VTEC may also be referred to as Shiga-like toxin producing *E. coli* (SLTEC) or Shiga toxin producing *E. coli* (STEC) because the verocytotoxin is closely related to Shiga toxin produced by *Shigella dysenteriae* type 1. They may also be referred to as enterohaemorrhagic *E. coli* (EHEC). In addition, not all isolates of *E. coli* O157 are verocytotoxic, although they frequently are (*Report on Verocytotoxin-Producing Escherichia coli* (1995)).
- 2.5 For the purpose of this report the focus will be on verocytotoxin-producing *E. coli* (VTEC) research and not the other pathogenic strains of *E. coli*.
- 2.6 However, there is a necessary caution. Researchers are not always consistent in their use of the terms VTEC, EHEC, *E. coli* O157, etc. As a consequence, there are occasions in this report where it is necessary to refer to *E. coli* O157 rather than to VTEC because it is not clear whether the specific organism described in the research project is in fact a VTEC. Furthermore, there are occasions within the report when it is necessary to refer to other strains or types of *E. coli*.

### 3. METHODS

- 3.1 This report is based, as was the 1999 MSFFG VTEC Report, on those projects which are funded by the members of the MSFFG. These are the Food Standards Agency (FSA), the Department for Environment, Food and Rural Affairs (Defra), the Biotechnology and Biological Sciences Research Council (BBSRC), the Department of Health (DH), the Department of Agriculture and Rural Development, Northern Ireland (DARD), FSA Scotland, FSA Wales, FSA Northern Ireland, Scottish Executive Environment and Rural Affairs Department (SEERAD) and Scottish Executive Department of Health (SEDH).
- 3.2 The MSFFG project database<sup>8</sup> was used to identify projects for inclusion in this report. The VTEC related projects were found by searching the database for Organism 'EHEC'. Additional projects were found by searching an extracted list of project titles for 'VTEC' and '157'. Projects which were completed by 1<sup>st</sup> August 1999 were not included. This gave a total of 103 projects for inclusion in this report: a full list of the projects is provided at Appendix 2.
- 3.3 Studentships have been omitted from consideration.
- 3.4 Research funded by other agencies, including the Wellcome Trust, Royal Society, NHS Scotland, Health Protection Agency (HPA) and the Medical Research Council (MRC), as well as international research is not included

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<sup>8</sup> The MSFFG maintains a database containing information about research projects in the area of the microbiological safety of food that are funded by the members of the MSFFG. Members of the Group provide the project information from their respective project record systems.

within the body of the report. However, a summary of research funded through these bodies is given in section 4 below.

#### 4. RESEARCH FUNDED BY OTHER FUNDING BODIES

##### Within the UK

- 4.1 Within the UK the vast majority of research on pathogenic *E. coli* is funded by the BBSRC, FSA and Defra. The Wellcome Trust funded a small number of projects on enteropathogenic *E. coli* between 1998 and 2002. These were generally genetic studies, addressing issues of molecular regulation and pathogenicity. In addition, the Wellcome Trust has funded an IPRAVE project<sup>9</sup> which is providing significant insights into aspects of the epidemiology of *E. coli* O157 as well as other organisms. The MRC records one *E. coli* grant within its Current Research database<sup>10</sup>, but this was not on VTEC. However, this database does not cover more recent research.

##### Within Europe

- 4.2 Through the Framework 5 programme, the European Union is supporting over 180 research projects on *E. coli*<sup>11</sup>. However, only six of these can be identified as being on VTEC or other pathogenic *E. coli*. Within this small group, the projects essentially cover all the areas described within this report. An important project was the now completed VTEC Concerted Action Project (CT98-3935) which brought together VTEC researchers across the EU, the USA and Australasia to assess and summarise best practice, leading to the publication of an overview of all the recommendations from the project: A European Study on Animal Food and Biomedical Aspects of *E. coli* O157:H7 (Duffy, Garvey and Sheridan (2002)<sup>12</sup>).
- 4.3 The EU has also funded Enter-net<sup>13</sup>, to monitor the occurrence of human VTEC and Salmonella infections. The network involves all twenty five EU member states (as of May 2004) plus several others<sup>14</sup>.

##### Within the USA

<sup>9</sup> International Partnership Research Award in Veterinary Epidemiology: Epidemiology and evolution of Enterobacteriaceae infections in humans and domestic animals. [www.vie/gla.ac.uk/wiprave](http://www.vie/gla.ac.uk/wiprave). The award is for over £3m from 1999 for five years, involving over 30 researchers in Scotland, Europe and North America.

<sup>10</sup> This is the publicly available database. Discussion with MRC staff has not led to any further projects being identified at this time.

<sup>11</sup> <http://www.cordis.lu/en/home.html>

<sup>12</sup> <http://www.teagasc.ie/research/reports/foodprocessing/4545/eopr-4545.htm>

<sup>13</sup> [http://www.hpa.org.uk/hpa/inter/enter-net\\_menu.htm](http://www.hpa.org.uk/hpa/inter/enter-net_menu.htm)

<sup>14</sup> Bulgaria, Canada, Iceland, Japan, Norway, New Zealand, Romania, South Africa and Switzerland.

- 4.4 The National Institutes of Health database of funded research<sup>15</sup> records very large numbers of funded research projects on *E. coli*, with only a relatively small subset (about 40) of these addressing verocytotoxic *E. coli* or associated EHEC. Most of these research projects focus on EHEC in relation to man, in particular colonisation, pathogenicity and virulence; host response; subsequent disease; the possibility of prevention or reduction (very few projects) and some work on detection. Some further projects, including about 10 addressing EHEC in cattle, are recorded in the Food Safety Research Information Office database<sup>16</sup>.
- 4.5 In contrast with research in the UK (see section 5 below), there is considerable effort in the US to develop multilocus sequencing typing (MLST) as the preferred method of typing the many serotypes of *E. coli*<sup>17</sup>.

#### A general overview of international research on VTEC

- 4.6 VTEC is recognised internationally as a research topic of importance in its own right: VTEC 2003, the 5th International Symposium on Shiga Toxin (Verocytotoxin) Producing Escherichia coli Infections, held in June 2003, provided an excellent overview of the effort in this area internationally, showing that there is substantial research effort in the US, EU and UK as well as in Australasia. Internationally there is significant research into the epidemiology of VTEC, including non-O157 VTEC in man, cattle and the food chain. The biology of the organism, essentially viewed from a molecular and genetic perspective, is also a major area of study. This research is benefiting from the availability of the *E. coli* O157:H7 genome sequence (Perna et al (2001)), and the current general focus on identifying genes and gene function.
- 4.7 The EU-funded Concerted Action project, has provided a coherent overview of research and the management of VTEC (Duffy, Garvey and Sheridan (2002)<sup>18</sup>). It deliberately considered VTEC from the perspective of the public health issues, and summarised important aspects of relevance to the food industry as well as government and private practices.
- 4.8 An issue with interpreting the research effort in the area of VTEC is the proliferation of forms of pathogenic *E. coli*, with different pathologies. In many ways, the VTEC research community divides into those who are interested in the fundamental science of the organism, be this the function of novel genes, the mode of action of virulence determinants, or the serotype of strains found in epidemiological studies; and those researchers who are involved in the practical issues of the management, control and (ideally) reduction of the levels of VTEC encountered by man. The majority of current research is in the former category, but the latter is

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<sup>15</sup> <http://crisp.cit.nih.gov>

<sup>16</sup> <http://www.nal.usda.gov/fsrio/index.htm>, <http://peaches.nal.usda.gov/fsrio/fsrioform.asp>

<sup>17</sup> <http://foodsafety.msu.edu/Whittam>, <http://www.shigatox.net/stec/mlst/>

<sup>18</sup> <http://www.teagasc.ie/research/reports/foodprocessing/4545/eopr-4545.htm>

clearly of great importance in terms of public health, strategic need and policy formation.

## MSFFG FUNDED PATHOGENIC *ESCHERICHIA COLI* RESEARCH 1999 TO THE PRESENT

### 5. DETECTION, DIFFERENTIATION AND DIAGNOSIS

5.1 Projects grouped into this research category address a number of issues. There is the issue of detection of pathogenic *E. coli* from a variety of different sample contexts, laboratory, man and animal. Having detected *E. coli* in the sample there is the issue of differentiating between the large number of serotypes of the organism. Here, the possibilities offered by new technologies, and the knowledge of the genome sequence of several different *E. coli* strains offer real potential to develop powerful and objective tools and methods. There is also a need for immunological methods for detecting past exposure of individuals to relevant pathogenic *E. coli*.

### 5.2 Overview of current research

#### Detection

5.2.1 *E. coli* is found in a wide variety of non-faecal materials as well as in faeces. Currently, routine detection of EHEC is by immunomagnetic bead separation followed by culture and identification of the specific serotype. The existing method is slow and labour intensive, as well as being able to handle only a limited number of samples. There is also an ELISA method, but this has limited sensitivity.

5.2.2 Improvements to the immunomagnetic separation methods for isolation of *E. coli* O157 have been achieved (**SEERAD UAB/005/97**) giving a more sensitive method able to recover VTEC from a high background level of competing organisms.

5.2.3 Several projects have been investigating methods and approaches to detecting VTEC in food, with the intention of devising methods which are both faster than the standard culture methods and more able to detect the presence of serotypes additional to *E. coli* O157 (**FSA B09007, B09008, B09010, B11003, B11004, SO FS0041**). Three of these projects (**B09007, B09010, B11003**) sought to use antibodies to detect the presence of verocytotoxin or bacterial antigens as a component of the assay. It was found that a successful dip-stick assay could be developed, using antibodies to verocytotoxins 1 and 2. In small-scale trials this method reliably detected *E. coli* O157:H7 directly in deliberately contaminated food and milk samples. There was also detection of *E. coli* O26, but with a lower success rate (**B09010**). Separately, it was found that a variety of immunoassay kits could detect verocytotoxin in VTEC enrichment cultures and that verocytotoxin extracted from food samples could be detected by two of the kits (**B09007**). An attempt to obtain

suitable monoclonal antibodies to bacterial antigens was unsuccessful, possibly because of inconsistent expression of the antigens (**B11003**). Further work within this project suggested that there were suitable VTEC O157-specific antigens which could be used in monoclonal antibody generation.

- 5.2.4 Research has also been undertaken to use molecular biology-based techniques in the detection of VTEC. Work in project **B09007** assessed a commercial Taqman® assay system and a standard PCR method. It was found that both methods were suitable for use with enrichment cultures of VTEC, and, for economic reasons, the standard PCR method was probably more suitable. However, there were still several issues to be addressed, in particular in methods for selectively enriching for specific serotypes. Work in project **B09008** showed that the levels of bacteria could be successfully enriched prior to PCR detection assay and addressed a number of problems with the inhibition of bacterial growth in a variety of foodstuffs.
- 5.2.5 Within a project to determine the incidence of VTEC in animal feedstuffs, specific assays to detect VTEC bacteriophage have been developed. These are effective in assaying feed samples including forage (**Defra OZ0711**). Similarly, in the process of confirming effective depuration methods for shellfish with respect to VTEC, methods for isolating and detecting the bacterium were developed (**FSA B04008**).
- 5.2.6 Further detection methods for *E. coli* O157 obtained from the environment, in this case from soil, have been developed as part of an investigation into water supply contamination. The system is PCR-based, and has been shown to give rapid (within one working day) identification of the presence of *E. coli* O157:H7 in soil and water samples (**SO UAB/007/99**).
- 5.2.7 A survey of over 300 companies undertaking or associated with microbiological tests on food found that the vast majority used conventional culture methods. Of the 208 involved in the food industry, only 58 laboratories were testing for *E. coli* O157 in-house, with a further 68 sending samples to another laboratory (**FSA B09005**). A separate survey found that the microbiological sampling and testing methods in use were diverse (**FSA M01017**), but further work is yet to be reported.

### Differentiation

- 5.2.8 A large number (over 170, see Report on Verocytotoxin-Producing *Escherichia coli* (1995)) of serotypes of *E. coli* have been identified using serotyping methods. These methods are variable in outcome due to issues of reproducibility and operator expertise. Furthermore, they do not necessarily discriminate adequately between different types of VTEC and EHEC. In the 1999 MSFFG VTEC Report, it was observed that sufficient research on the typing of VTEC had been undertaken. However, there have subsequently been further developments in molecular biology

techniques, including the availability of the *E. coli* O157:H7 genome sequence (Blattner *et al* (1997)) which underpin the continued investigation of a number of novel typing and classification approaches.

- 5.2.9 Methods investigated include the use of amplified fragment length polymorphisms (AFLP) (**FSA B01014**), reverse-transcriptase PCR (RT-PCR) (**Defra OZ0704**), PCR (**FSA B14004**) and whole-genome PCR (**BBSRC D13414**) DNA microarrays (**Defra VF0101**), pulse field gel electrophoresis (PFGE) (**SEDH k/mrs/50/c2624**, **Defra OZ0138**) and genotyping using bacterial interspersed mosaic elements (BIMEs) (**BBSRC BFP11335**). In addition, one project has noted that the growth of *E. coli* O157 on different media may be more discriminatory than conventional phage typing, but no further work has been undertaken (**SEERAD UAB/005/97**).
- 5.2.10 AFLP methods are used widely in the differentiation of bacterial types, and the methods are being applied to VTEC to develop a robust, reproducible assay, with significant attention being given to the needs of inter-laboratory reproducibility (**B01014**). However, this method does not appear to have been applied to any of the collections of strains available (**B01014**, **B14004**).
- 5.2.11 BIMEs have been shown to have significant potential in genotyping *E. coli* strains and these have been used to investigate genetic diversity in VTEC and other *E. coli* isolates and to compare this with the genetic variation observed using phenotypic characterisation methods (**BFP11335**). Both approaches found that there was extensive genetic variability in EHEC strains, but that O157 showed a lesser degree of variability than some others.
- 5.2.12 RT-PCR is being proposed as a route to identifying virulence factors expressed by *E. coli* O157:H7 (**OZ0704**). Similarly, DNA microarray technologies are being developed to study a range of EHEC and related enteropathogenic *E. coli* (**VF0101**), and this approach might provide a broadly applicable differentiation technique.
- 5.2.13 The whole-genome PCR is being used primarily to explore the genomic diversity found amongst enteric bacteria, with *E. coli* as one of the foci of the project (**D13414**). It is an aim of the project for the methods developed to be sufficiently robust, simple and cheap that they could be used for routine molecular epidemiology and research into the pathogenicity of bacterial species. Importantly, another molecular technique, PFGE, is reported as being able to differentiate between outbreak-associated *E. coli* O157 and sporadic strains (**k/mrs/50/c2624**). This was observed when undertaking real-time investigations of outbreaks in the field, exploring the transmission of *E. coli* O157 from dairy cattle to man.
- 5.2.14 Determining whether pathogens such as *E. coli* O157 are resistant or sensitive to environmental stress is valuable when investigating the movement of such pathogens through the food chain. Tests based on

colony growth on solid media were found to be useful in differentiating between bacteria that were tolerant or sensitive to stresses such as acid, heat, air-drying and peroxide (**FSA B01007**). The tolerance of various bacterial strains, including *E. coli* O157, to these stresses was inherently variable, requiring comparison between strains before further research was undertaken. Distinguishing between live and dead bacterial cells in the food chain may also be of significance, and there is work to develop analytical methods for such organisms isolated from food (**SEERAD URG/001/96**).

### Diagnosis

- 5.2.15 There is a need to be able to diagnose the current or previous presence of VTEC even in the absence of the pathogen itself. Using a commercially available saliva sampling kit, work has shown that human saliva samples can be collected and antibodies to *E. coli* O157 detected, including after transport of the samples to the laboratory (**FSA B11008**). Assay of saliva samples from healthy adults (52) and children (425) did not find antibodies in any samples, suggesting that false positives would probably not occur. Disappointingly, antibodies were not detected in saliva in more than 45% of samples from patients with *E. coli* O157 antibodies in their blood: this was believed to be due to the timing of the samples and the production of antibodies not coinciding. Antibody-based assays are also being developed to enable investigation of human response to *E. coli* O157 and the management of disease (**SEERAD k/mrs/50/c2624**).
- 5.2.16 Work is also being undertaken to develop a simple field assay which could detect VTEC in the terminal rectum of cattle, the principal site of colonisation (**Defra OZ0712**).

### 5.3 Gaps in currently funded research

- 5.3.1 In the 1999 MSFFG VTEC Report, it was noted that in the areas of detection and differentiation there were significant needs for:
- a simple test for detection of VTEC which could be widely used
  - a method for detection of all VTECs and not just *E. coli* O157.
- 5.3.2 Methods have now been established for the detection of VTEC O157 based on serotyping and phagetyping]. However, these approaches are not the best methods for identifying non-O157 subtypes, and there remains a need to develop and agree a method for detecting all VTECs, and other pathogenic *E. coli*. Some progress in this area is being made through the VTEC consortium (**B09007**, **B09008** and **B09010**).
- 5.3.3 The 1999 MSFFG VTEC Report also identified the need for a non-invasive diagnostic test for use in man. Some exploration of such an assay has been undertaken, but if one is still needed, significantly more work will be

required.

- 5.3.4 Considering the issues identified in the previous report, and the research that has subsequently been undertaken, the principal gaps would appear to be:
- the need for an agreed, validated and robust set of isolation and identification protocols for use in research projects (and elsewhere) involving the detection of a wide variety of serotypes of *E. coli*.
  - the need to determine how the current typing schemes relate to the *E. coli* genome sequences and other genetic information now available, including the genomic method used for *E. coli* O157.

## 6. MICROBIAL PHYSIOLOGY AND GENETICS

- 6.1 *E. coli* is undoubtedly the most extensively studied bacterial species in terms of its biochemistry, physiology and genetics. It has provided the model and basis for research into many other bacteria, and the depth of knowledge of its genetics undoubtedly contributed to the development of techniques which were essential to biotechnology (eg Cohen and Boyer (1980)). The genome sequence of the type organism, *E. coli* K12 MG1656, was published in 1997 (Blattner et al, (1997)), providing a further essential tool in *E. coli* genetics. Subsequently, genome sequences for pathogenic strains of *E. coli* have been published (Perna et al, (2001)<sup>19</sup>) providing the opportunity for identification of genetic variation between the different strains, and exploration of the underlying genetics and physiology which lead to pathogenicity in specific strains of the organism.
- 6.2 The study of the genetics and physiology of *E. coli* seeks to develop an understanding of how the organism functions in a given environment; how the genetic make-up of a particular strain varies from other strains with different environmental and host interactions; and what are the underlying characteristics of the organism which may explain its behaviour. The availability of the genome sequence of the type strain, *E. coli* K12 along with that of *E. coli* O157 and other pathogenic Enterobacteriaceae has provided new impetus to this work, particularly for mechanisms of virulence and pathogenicity (Defra OZ0707, Kaper, Nataro and Mobley, (2004)). Further impetus will be given with the availability of the genome sequence for *E. coli* O42, an enteroaggregative *E. coli* currently being sequenced at the Sanger Institute, as this will enable better comparison between different types of *E. coli*.

### 6.3 Overview of current research

#### Technologies and tools

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<sup>19</sup> <http://www.genome.wisc.edu/sequencing.htm>

6.3.1 The release of the various *E. coli* genome sequences has enabled researchers to develop a number of tools which exploit this information. These include DNA arrays (**BBSRC EGA16107, Defra VF0101**), proteomics (**BBSRC EGA16107, BFP11341, Defra OZ0705**) and the use of signature-tagged transposon mutants (the overall method being known as signature tagged mutagenesis, STM) to identify genes which are essential in virulence of VTECs and other *E. coli* (**VF0101, BBSRC D14378, Defra OZ0707**). These methods underpin much research not only into the genetics and physiology of VTECs but also into other aspects, in particular possible properties required for colonisation and infection by VTECs of different hosts. In addition, a reverse genetic approach was used to develop a system for targeted gene disruption in *E. coli* O157 and related bacteria (**Defra OZ0705**). Methodologies have also been developed for the identification of gene function from cellular metabolites (**BBSRC G11549/12163**).

#### Gene function

6.3.2 STM is being used to explore a number of aspects of gene function. For example, work within project **OZ0707** identified over 80 genes which are implicated in intestinal colonisation of 10- to 14-day old calves. A number of these genes were located to the 'O-islands': clusters of genes which are specific to *E. coli* O157:H7 and not found in *E. coli* K12. In addition, there were significant numbers of genes of unknown function. Recently an investigation of gene clusters in *E. coli* O157 containing putative adhesin genes which are not present in non-pathogenic strains (**BBSRC D15913**) has been initiated.

6.3.3 Adhesins are important in the process of colonisation and infection of hosts by VTEC. VTEC strains were analysed to identify the presence of *eaeA* and other adhesion associated genes and *eaeA* mutants were tested in adhesion assays (**FSA B11006/B11007**). It was found that adherence of *eaeA* negative mutants was the same as wild type adherence, suggesting that the mutants had an alternative mechanism for adherence. It was also noted that the distribution of VTEC when adhering to abiotic surfaces was strongly influenced by the charge of the surface (**B11006/B11007**). Pathogenic *E. coli* generally have the capacity to express a variety of adhesins and the control of expression of these is being investigated, along with exploration of the impact of environmental conditions on this regulation (**BBSRC P10995**).

6.3.4 Also of importance in the mechanism of infection by VTEC is a type III secretion system (TTSS): these are common amongst Gram-negative pathogens. The unique TTSS found in VTEC is being studied (**BBSRC D17455**) and so far the structure and function of the components of the secretion mechanism have been characterised.

6.3.5 Not surprisingly, there are a number of projects in which the genetic variation between various strains of *E. coli* is being investigated (**Defra OZ0707**). It was found that, although all EHEC strains showed substantial

genetic variation, there appeared to be less variability within *E. coli* O157 than other EHEC serotypes (**BFP113355**). Using a proteomics approach, it was observed that there were few differences between field and clinical isolates of EHEC O157, and that such variants as were observed were due to isoforms of the same proteins (**Defra OZ0705**).

### VTEC and its environment

- 6.3.6 It was found that a variety of *in vitro* environmental conditions influenced the adherence of VTEC to two different cell lines, HeLa and Hep-2 (**FSA B11005**). In particular it was found that actively growing cells were more adherent than those in stationary phase and that the pH of the growth culture was important, with maximum adherence being observed between pH 6 and 7. Anaerobic culture conditions were found to lead to even greater adherence of the VTEC, and VTEC grown in media with reduced levels of iron were shown to adhere less than bacteria grown in standard medium.
- 6.3.7 The effect of acidic conditions and reduced temperature on the survival capacity of VTEC was investigated (**B11006/B11007**): it was found that prolonged culture in minimal media at 8°C reduced the viability of the bacteria. Work was also undertaken to explore the adaptation of food-borne pathogens, including *E. coli* O157, to environmental conditions. The mechanisms of adaptation, such as genetic change and gene regulation are being explored (**BBSRC 3258371**).

## 6.4 Gaps in currently funded research

- 6.4.1 In the 1999 MSFFG VTEC Report, it was noted that gaps in the understanding of the physiology of VTEC were being addressed. Despite this, there does not seem to have been significant progress in the general understanding of the physiology of VTEC. This may in part reflect that research into the physiology of *E. coli* is not focussed on VTEC but covers a much wider range of strains of *E. coli*<sup>20</sup>. In particular, in the area of microbial physiology and genetics, the emphasis is likely to be on the identification of genes of unknown function and subsequent determination of their function. This is a reflection of the impact of the availability of the genome sequences. Interestingly, though, there does not appear to be substantial effort comparing the genome sequences of *E. coli* K12, *E. coli* O157 and other strains of *E. coli*. The opportunities for comparative genomic approaches will be greatly increased once the further *E. coli* genome sequences currently being obtained are complete<sup>21</sup>. In this context, it is worth noting the observation in **Defra OZ0702** that non-O157 strains of *E. coli* are not appropriate experimental models for *E. coli* O157.

<sup>20</sup> For example, the BBSRC Oasis database finds 189 projects using the search term *Escherichia*, 251 projects using *E. coli*, 17 using O157 and 2 using VTEC.

<sup>21</sup> [www.sanger.ac.uk](http://www.sanger.ac.uk)

- 6.4.2 In the 1999 MSFFG VTEC Report a number of environmental conditions and circumstances were noted for which it was felt there would be benefit in developing an understanding of the behaviour of VTEC. There remains a lack of information on how *E. coli* O157 survives in a quantifiable manner in the various stages of the food supply chain, including the preparation, preservation and treatment of food. Developing this understanding is still required.

## 7. PATHOGENESIS AND HOST RESPONSE

- 7.1 For a gastrointestinal pathogen such as VTEC, introduced to man through the food chain and present in a non-disease-causing form in farm animals, the pathogenicity of the bacterium covers a number of issues. Although the general process of VTEC pathogenicity is well known, there is still much to determine in the detailed mechanism. Overall, the process involves the adhesion of the organism to the intestinal tract of the host, followed by successful colonisation and the production of toxins (the verocytotoxins) which then act on the cells around the site of infection. Research into all these aspects of VTEC pathogenicity is addressed in this report, with a particular focus on laboratory investigations of the mechanisms by which the bacterium adheres to the epithelium of the intestinal tract. This includes the colonisation of animals which do not show symptoms as well as invasion and virulence in man.

### 7.2 Overview of current research

#### Genetic and proteomic studies of the causes of VTEC pathogenicity

- 7.2.1 Using signature tagged mutagenesis (STM), over 80 mutants which are defective for intestinal colonisation of calves have been identified (**Defra OZ0707**). These include mutants with insertions in: the locus of enterocyte effacement (LEE); the LEE-encoded type III secretion apparatus; 'O-islands', including an insertion proximal to a putative iron-regulated adhesion, a gene encoded by the Type I fimbriae-like operon; and a large number of hypothetical genes which logically must be involved in invasion and colonisation by the organism of calves. Similar STM studies of clinical isolates are being undertaken in project **Defra VF0101**, and further *in vitro* mutant studies to determine the molecular basis of the colonisation of the terminal rectum of cattle are proposed in project **BBSRC D19613**.
- 7.2.2 Using proteomic techniques, the BipA regulator protein has been found to have a key role in the expression of motility and secretion via the locus of enterocyte effacement (LEE): both properties are key virulence factors (**Defra OZ0705**). However, mutants with defective genes in the cryptic secretion system (which is associated with virulence in *Salmonella*) did not show significant differences in protein expression compared with the parent strain, suggesting that this mechanism was not functional (at least

under those growth conditions).

- 7.2.3 VTEC and other pathogenic *E. coli* have the capacity to produce a variety of adhesins, but these are not generally produced at the same time. The cross-regulation of the various adhesin gene clusters, and the effect of the environment in this cross-regulation, has been investigated (**BBSRC P10995**). Additionally, by comparison of the *E. coli* K12 and *E. coli* O157 genomes, five putative adhesin gene clusters present in the pathogen but absent from the wild type organism have been identified. Whether these genes express functional adhesins, and what their role is, will be evaluated, primarily in relation to bovine cells (**BBSRC D15913**).
- 7.2.4 It has been noted (eg **FSA B11005** below) that outer membrane proteins of VTEC are implicated in virulence of the organism. One of these, Antigen 43 (AgA43), is particularly implicated in virulence and is the subject of further study to determine its role in infection and colonisation (**BBSRC D14955**).
- 7.2.5 In the search for genes which are involved in pathogenicity of VTEC, comparative studies with non-O157 EHEC have found a homologue of LifA, which is involved in colonisation of calves by non-O157 EHEC, in *E. coli* O157. The role of this gene is being studied, alongside the LifA gene and gene product, lymphostatin (**BBSRC D17455**).

#### Verocytotoxin

- 7.2.6 An absolute characteristic of VTEC is the production of verocytotoxins, which are expressed from the VT genes encoded on VT-bacteriophages which then reside within the chromosome. However, the phage are lysogenic and can therefore cross-infect from one strain of *E. coli* to another. Research has shown that a tagged VT phage infected a wide variety of *E. coli* strains from human and livestock sources; in addition, wild type strains (ie non-pathogenic) with an intact lipopolysaccharide coat could also be infected (**BBSRC BFP11345**). The mechanism by which the VT phage infected *E. coli* was found to include an outer membrane protein Vpr. The gene for this was found to be widespread amongst *E. coli* strains.

#### Physical and other related studies

- 7.2.7 In exploration of whether adherence was achieved through fimbriae, a number of VTEC strains were investigated by electron microscopy after appropriate staining to reveal the structures (**B11005**). None of the VTECs examined showed fimbriae, leading to the conclusion that the mechanism of adhesion for these strains was through non-fimbral adhesins. The same strains were then investigated by seeking to block adhesion to cells with a variety of bacterial components including lipopolysaccharide (LPS), outer membrane proteins (OMPs) and antibodies specific to O157 antigen, OMPs and H-7 flagella. It was found that OMPs gave the highest degree of inhibition of adhesion for the VTEC

strains. The expression of these proteins under conditions which had been found to give increased and decreased levels of adhesion was then investigated and it was observed that there was variation in specific proteins under the various conditions. It was suggested that this variation in OMP expression was a response of the bacteria to specific environmental stresses which in turn might mediate bacterial adhesion to mucosal surfaces. Similarly, it was found that, although most non-O157 strains of VTEC expressed type 1 fimbriae, O157 VTEC expressed neither curli or type 1 fimbriae under the same conditions (**FSA B11006/B11007**).

7.2.8 The three dimensional structure of *E. coli* O157 cell-binding domains is being investigated using NMR as a route to understanding the mechanism(s) by which the bacteria bind to host cells (**BBSRC BFP11329**). In addition, electron microscopy will be used to investigate the structure of the type III secretion system in both EHEC and EPEC (**BBSRC B17144**).

7.2.9 Microscopic examination of human cell cultures infected with VTEC has found that the strains show two different adherence mechanisms (**B11005**). With HeLa and Hep-2 cells, the VTEC bind to the extracellular microfilaments, whereas the same VTEC strains appear to be invading the Caro-2 and HT-29 cells. Using gentamicin survival assays, it was confirmed that *E. coli* O157 was only adherent to Ht-29 and Caro-2 cells.

#### Secretion systems

7.2.10 Secretion systems are also a component of the pathogenicity of VTEC. It has been found that proteins secreted by the LEE-encoded Type III secretion system are important to the successful intestinal colonisation of calves by *E. coli* O157 (**Defra OZ0707**). However, it was not clear that a particular protein secreted through this mechanism, translocated intimin receptor, was as significant.

#### Research in animals

7.2.11 Alongside the extensive genetic studies into the pathogenicity of VTEC, there is also work to consider virulence *in vivo*. One need is for an assay to identify and quantify *in vivo* the virulence factors produced by *E. coli* O157, and work has been undertaken based on RT-PCR (**Defra OZ0704**) in this area.

7.2.12 Although many genes have been identified at some level as being implicated in bovine, and other farm animal, colonisation, there is a need to confirm this both through mutant studies and by examination of environmental and clinical isolates of VTEC. Some components of the latter will be addressed through project **VF0101**, which intends to apply STM technology to identifying genes and gene products of *E. coli* O157 which are required by the organism for colonisation of cattle. This will be

complementary to work already undertaken in **OZ0707**.

- 7.2.13 It has been observed in some cattle that *E. coli* O157 is primarily located in the colon, rather than being distributed throughout the intestinal tract (**Defra OZ0702**) with, for some cattle, the principal location being the final few centimetres of the terminal rectum (**Defra VF0304**). This latter affinity may be in part be due to a lack of expression of various adhesins, which have acquired mutations preventing their normal expression or activity: for example the Type III secretion adhesins (EspA and intimin) are switched off in the majority of the bacteria moving through the gastrointestinal tract. In addition, it is suggested that there is adhesin-binding to receptors in the terminal rectum which are unique to that region. For the bacteria to colonise this region also requires that there is limited endocytosis by the epithelial cells: research suggests that the Type III secretion apparatus is functional at this stage of the colonisation process and leads to the injection of virulence factors into the host cells which then disable them. This is coupled with the expression of intimin which enables bacterial attachment to the host cells. The project has obtained further evidence, all of which supports the view that the host cells fail to mount an immunological response to the bacterial colonisation at this point in the rectum, enabling long-term persistence of the bacteria and both host re-colonisation as well as exposure of other animals in the herd (**VF0304**). Similar issues of the colonisation of the terminal rectum of cattle are being explored though a combination of *in vitro* mutant studies and characterisation of the relevant components of the gastrointestinal tract of cattle (**D10613**). There is also a recently funded project which seeks to understand the colonisation of the terminal rectum from a whole organism perspective, and then to examine various routes to elimination of the bacteria (**Defra OZ0712**).
- 7.2.14 Although cattle are regarded as the primary source of VTEC infections in humans, there is concern that the pathogens are also being introduced to the food chain from goats, pigs and sheep. Work is under way to explore the role of goats and pigs: initial results have already shown that *E. coli* O157 successfully colonises the large bowel of pigs, with the highest bacterial counts being found in the colon and proximal rectum. In goats it was found that neonatal animals were susceptible to colonisation by *E. coli* O157 (**Defra OZ0710**).
- 7.2.15 The colonisation and pathogenicity of VTEC in sheep is being investigated (**Defra OZ0706**): the organism appears to colonise the animals (in that there is long-term persistence of the bacteria) but in sheep of various ages it does not give the attaching and effacing (A/E) lesions seen in cattle.

#### Studies in humans

- 7.2.16 An *in vitro* organ culture model of the human intestinal mucosa has been developed in order to explore the interaction between EHEC, and *E. coli* O157 in particular, and the human host. It has been observed that there is colonisation by *E. coli* O157 of specific cells (the Peyer's patches) to form

the A/E lesions (**BBSRC D10262, D10261, 0754**), and further studies are planned on the impact of toxins, the initial events in colonisation and to identify novel virulence factors (**BBSRC D13600**).

### 7.3 Gaps in currently funded research

7.3.1 In the 1999 MSFFG VTEC Report it was noted that there were many, and significant, gaps in the existing understanding of the mechanisms of pathogenicity of VTEC. Of particular concern was the lack of work to relate the understanding of the bacterium's pathogenicity in man to the rather different behaviour in animals. Specific concerns which were identified were the need to understand:

- the attaching and colonisation mechanism in colonised, asymptomatic calves and how this compared to the findings in humans,
- the reasons VTEC adhere to some species and not others,
- the role of virulence factors and, in particular, the role of the toxin in the disease process,
- the significance of antibody production and the dynamics of the antibody reaction,
- the significance of factors such as the human diet in relation to the virulence of the disease, e.g. the acquisition of iron and its role in the disease process,
- resistance to VTEC in humans and the role of asymptomatic carriers, particularly in relation to age and dose.

7.3.2 In addition, the 1999 MSFFG VTEC Report noted that there could be a need to develop an animal model to represent human disease.

7.3.3 Considering the substantial work described in this report relating to the understanding of VTEC pathogenicity in man, and colonisation of farm animals, significant progress has been, and is continuing to be, made in these areas, especially in knowledge of the mechanisms of colonisation of cattle.

7.3.4 An area where there appears to have been very little progress, or focus, is that of the relationship between the human host and the organism. The 1999 MSFFG VTEC Report identified issues such as diet, human resistance to VTEC and the role of asymptomatic carriers as being of significance, but there appears to have been no research in these areas supported by the MSFFG members. The issue is whether this area is one of fundamental importance, or whether greater research benefits will be obtained by focusing on the colonisation of farm animals and the environment and then developing mechanisms for the reduction and elimination of the organism from these sources of infection.

7.3.5 There is also no research recorded relating to the human immunological response to VTEC and other *E. coli* strains. It is likely that this is important

as, for example, VTEC infections are more common and have more serious consequences in the very young and the elderly (**DH 247**).

7.3.6 One issue which may need to be considered is the relationship between the growth environment of VTEC in the laboratory as compared with the conditions the bacterium encounters *in vivo*. These will be significantly different and are likely to have a major impact on the properties of the organism. Some research in this area might be valuable.

7.3.7 An area where there is visibly substantial progress, as already noted (section 7.2.1 and following), is in the infection and colonisation of farm animals, in particular cattle. There would seem to be two issues; one is to ensure that there is not excessive duplication of effort; the other is to ensure that the research findings are brought together to develop a coherent model which explains the overall process and informs work on the reduction and elimination of VTEC from the food chain.

## 8. EPIDEMIOLOGY

8.1 Study of the epidemiology of VTEC includes research into the occurrence of the organism in farm animals, the environment, in food and in man. It should consider the transfer of VTEC between these different hosts and contexts, and how the organism becomes established within them. As part of this work, epidemiological studies would be expected to consider the occurrence of different types of VTEC and the movement of such types between hosts.

### 8.2 Overview of current research

#### General

8.2.1 As part of the IPRAVE project, a modelling approach is being taken to explore aspects of the epidemiology of *E. coli* O157 from cattle through to man (**SEERAD-ABGR BSS/028/9**). The aim is to develop a mathematical and statistical approach to analysing *E. coli* O157 incidence data, leading to new tools for the understanding of VTEC infections in cattle and man.

8.2.2 The FSA is developing a risk assessment model for VTEC O157, *Development of a risk assessment model for the different pathways of infection by E. coli O157* (**FSA B01019**). This includes a significant review of the sources of VTEC, transmission to animals and man, routes of transmission and the prevalence of the organism. This project summaries, and extends, some of the work described in section 8.2 below.

#### Farm animals

8.2.3 A study of the patterns of faecal excretion of VTEC O157 (**Defra OZ0138**) found that 39% of the 75 cattle herds studied were positive for VTEC

O157, and that across all herds 4.2% of cattle were infected (10% within infected herds). The age of the animals was significant in relation to the levels of VTEC O157 which they excreted, with the highest shedders being 3-24 months. A longitudinal study was initiated, but this was stopped as a result of the Foot and Mouth Disease outbreak, although not before some evidence had been accumulated to suggest that the prevalence of the organism in faeces was highest in summer. This would be consistent with the observation (**FSA Scotland S01018**) that although *E. coli* O157 is more prevalent in cattle in winter, higher concentrations of the bacterium are shed in the summer.

- 8.2.4 In a separate study (**Defra VF0201**) 25% of 63 farms studied had animals positive for *E. coli* O157 and the highest prevalence of the organism was in young stock and beef animals. This was further supported by a longitudinal study over a 12-month period which found that *E. coli* O157 positive weaned and unweaned calves shed the greatest numbers of the organism. There was considerable variation on the shedding patterns with time, which did not appear to be affected by the season. A similar study in Scotland (**SEERAD SAC/168/99**) found that at least a quarter of farms had at least one shedding animal at the first visit by researchers.
- 8.2.5 Using BIME analysis (see paragraph 5.2.11), it was found that cattle within a herd tended to be infected with the same strain of *E. coli* (**BBSRC 11335**) and that these were not necessarily *E. coli* O157. This was supported by the finding (**VF0201**) that, among cattle herds investigated in detail, there tended to be consistent (over 12 months) dominant serotypes of VTEC in the faecal samples.
- 8.2.6 Further research has been undertaken to determine the presence and persistence of *E. coli* O157 in farm animals (primarily cattle) (**Defra OZ0133, MAFF FS0035**), in particular in Scotland (**SAC/168/99**). In the last of these projects it was observed that increased shedding was associated with housed rather than grazing animals. Farms which purchased stock for finishing (rather than breeding their own) were also more likely to have shedding animals. There is a project to determine the prevalence of non-O157 VTEC in finishing beef cattle in Scotland, also including serotypes *E. coli* O26, O103, O111 and O145 (**FSA Scotland S01014**). Alongside this, there is also research to determine whether there is any interaction between the populations of non-harmful *E. coli* and pathogenic VTEC in cattle (**SEERAD-ABRG UAB/006/97**).
- 8.2.7 The patterns of bacterial communities in faeces from cattle which were shedding VTEC were compared with those from non-shedding cattle from the same herd, in the anticipation that variation in the patterns, including with time, would provide information on the patterns of VTEC contamination of stock (**SEERAD SCR/507/97**). It was found that the bacterial communities so investigated were highly complex, with variable profiles both between cows and for an individual animal over time. There was no consistent profile associated with VTEC shedding.

- 8.2.8 The levels of bacterial contamination of carcasses is likely to be indicative of the levels of contamination of the livestock. Work has been undertaken to investigate whether carcass contamination levels are linked to livestock condition and animal husbandry (**SEERAD SAC/125/96**).
- 8.2.9 It is recognised that ruminants other than cattle are also likely to be carriers of VTEC. Whilst investigating the colonisation of goats of various ages by *E. coli* O157, it was noted that some of the kids were already colonised by other strains of *E. coli*. (**Defra OZ0710**).

#### Farms, the environment and abattoirs

- 8.2.10 Since the primary route of infection of any animal by another one is through faecal material, there is the possibility that VTEC will pass into the food chain through farm waste material and its use as fertiliser (**Defra WA0804, FSA B05003-4, SEERAD SAC/138/96**). In farms in Scotland, it was noted that larger farms and those which spread slurry were more likely to have herds which included animals shedding *E. coli* (**SAC/138/96**). Samples of livestock manures taken from a diverse range of locations in the UK showed that around 20% contained glucuronidase-negative *E. coli* (ie strains with a property typical of *E. coli* O157). It was found that after spreading of manure over pasture, there was a progressive decline in pathogen levels over a two month period, such that at the end they were below the limits of the detection by the assay. In contrast, where livestock had been grazed on fields, it took 8 months from the time of their being moved for *E. coli* levels to return to the background levels (**B05003-4**).
- 8.2.11 Research has found that anaerobic digestion or aerobic composting could reduce the survival levels of VTEC, and that most pathogens only survive in manure heaps for 8 days in storage heap experiments, although survival of pathogens in slurries stored in commercial-scale tanks was for significantly longer (**B05003-4**): VTEC could survive for up to 9 months on faecally contaminated surfaces, and in water for up to 38 weeks (including in water troughs) (**Defra OZ0709**). (One useful observation was that farm slurries and dirty water are almost always disposed of at the farm of origin, meaning that the risk of transmission of VTEC through this route to other farms was low.) In addition, VTEC could survive in poorly made-up cattle feed.
- 8.2.12 In separate research a similar duration of survival of *E. coli* O157 was demonstrated, with the organism persisting in soil, waste and freshwater for up to six months (**BBSRC D12282**). It was suggested that this ability to persist meant that it was likely that there could be long-distance transport of *E. coli* O157 via freshwater and aerial routes during waste spreading. Also, there could be contamination of groundwater supplies, particularly during heavy rainfall. This in turn could lead to the contamination of private water supplies. Although not a major source of water throughout the UK, such private supplies are more common in agricultural areas, including in Scotland. Projects have been funded to investigate this route of

transmission (**SEERAD SAC 204/98-UAB/007/99, Defra WA0804**). Results from **SAC 204/98-UAB/007/99** suggest that the greatest risk of pollution of water supplies is immediately after the slurry application and that there can be significant levels of *E. coli* in water drawn off from such land. Levels of *E. coli* were affected by the type of soil, with heavier soils leading to higher levels of contamination than sandy soils. It was noted that there is also evidence that grazing animals may represent a greater risk to drinking water than slurry application, and it was found that indeed shedding grazing animals did lead to a build-up of *E. coli* in soil which then leached into drinking water (**SAC 204/98-UAB/007/97**).

- 8.2.13 Abattoir wastes are also a source of pathogens, but a survey of such waste applied to agricultural land did not detect any *E. coli* O157. The reasons for this surprising result were not clear, and could include the increased levels of disinfectants in use because of the FMD outbreak, or the effect of storage on the bacteria (**FSA B05008**). Equally, a similar result was found (**DANI 9723**) when 210 faecal samples were assayed: only 2 were found to contain *E. coli* O157: project **S01018** also supports a relatively low level of contamination of abattoir material, reporting work from a previous project which found only 7% of cattle tested at abattoirs as being *E. coli* O157 positive.
- 8.2.14 The levels of *E. coli* O157 associated with sheep brought to abattoirs were also found to be very low, with only two carcass samples (out of 97) showing contamination with the bacterium (**FSA M01011**).

#### Man

- 8.2.15 It was found that the *E. coli* O157 genotypes found in man were present in a variety of animals, suggesting an infective link (**k/mrs/50/c2624**). Equally, there was also evidence of recurrent clonal types of *E. coli* O157 which caused significant disease in man. There is also some evidence that there is an association between O blood group and severity of disease due to *E. coli* O157 in man (**Scottish Office Department of Health k/mrs/50/c2601**).
- 8.2.16 In the 1999 MSFFG VTEC Report, a number of case control studies were included, results from which had not been reported at the time (**DH241**, a study in England, **DH 247**, a study in Scotland, **DH254**, a study in Wales). It was found that the exposure to farm animals, non-domestic animal faeces, or being in contact with farms for work or domestic reasons (such as having a garden next to a field or farm) was significantly associated with a risk of infection with *E. coli* O157 (**DH 247**). Results from **DH 241** supported these observations but also indicated that other risk factors included contact with symptomatic, infected individuals, travel within the UK, eating out and exposure to recreational water. When considering people working and living in a rural context, the Welsh study noted that having a private water supply, handling raw salad and raw poultry were associated with excretion of VTEC (**DH 254**).

- 8.2.17 There was found to be both variation in the occurrence of infections in particular it was noted that more cases were reported from rural areas (DH 241). It was also found that amongst farm workers and people living in rural areas, the numbers with VTEC-containing faeces were lower during the winter than at other times of the year (DH 254).
- 8.2.18 It was noted that the most common routes of transmission of VTEC O157 were person-to-person spread, contact with animals and consumption of contaminated food (DH 241).
- 8.2.19 Infection with *E. coli* O157 was found in all three projects to be most common in individuals below the age of ten (over 40% in the two studies giving figures) and highest in children under five (DH 254).

### 8.3 Gaps in currently funded research

- 8.3.1 In the 1999 MSFFG VTEC Report, it was noted that considerable progress had been made in developing an understanding of the routes of transmission of VTEC to man. However, this in turn led to many questions which might need to be addressed, and were identified in the report. Of the various issues considered, there has been significant progress in understanding the prevalence of excretion of VTEC from cattle, and the nature of transmission through the environment. It is of note that VTEC contamination of carcasses at the abattoir is generally found to be low.

Thus, outstanding from the 1999 MSFFG VTEC Report were questions such as:

For humans

- Which route of transmission between/to people is most important?
- Are particular groups of people (eg farm workers) at an increased risk of VTEC infection?
- Why is there seasonal variation in the occurrence of infections?
- What is the effect of travel on the occurrence of infection, and is there a seasonal pattern to this?

For animals:

- What causes sporadic infections and what is the significance of non-food-borne infections?
- What were the routes of transmission of VTEC to food animals and other potential sources of the organism?
- Why is there geographical variation in the incidence of infection?
- Is there seasonal variation in incidence?
- What was the occurrence and prevalence of VTEC in wild animals, pets and poultry?

For all hosts and environments, there was also the issue of the epidemiology of nonO157 VTEC, as these were being implicated in human disease.

- 8.3.2 Many of these issues have been successfully addressed in the period since the 1999 MSFFG VTEC report. With respect to transmission of

VTEC to man, various research projects have increased the understanding of how this occurs, and have identified such risk factors as involvement with farms, either for work or recreationally. There remain areas where further research could be beneficial: for example although it is clear that travel within and outside the UK is a risk factor for VTEC infection, it is not clear how significant this is, nor whether it is related to routine or occasional travel. It is also unclear why there may be seasonal variation in infections in man.

- 8.3.3 Although there has been considerable progress with research into the epidemiology of VTEC in animals, there remain some areas where further research would be beneficial. These include: the causes of sporadic infections and the importance of non-food borne infections; the routes of transmission of VTECs to food animals and other potential points at which VTEC can be introduced into the food supply chain; the reasons for geographical variation in the incidence of infection: the occurrence and prevalence of VTEC in wild animals, pets and poultry.
- 8.3.4 The development of a risk assessment model for VTEC infection (**B01019**) emphasises that there is a need for further epidemiological research on the fate of VTEC in a variety of environments and how these affect entry of VTEC into the food-supply chain. It also notes that overall the greatest risk factor for infection with VTEC is exposure to animal faeces.
- 8.3.5 For all hosts and environments research is needed on the epidemiology of non-O157 VTEC, as these types of *E. coli* are also being implicated in human disease.
- 8.3.6 Many of the issues raised above are being addressed by current research (eg **FSA B01019**). However it is the case that much of the focus of VTEC epidemiology has been on animals, with less progress being made in understanding of human epidemiology. One reason for this has been the difficulty in pursuing the case control studies undertaken in the mid 1990s (**DH 241, 247 and 254**). This may be the result of declining interest in the illness, caused in turn by the declining occurrence of VTEC infections.

## 9. REDUCTION AND ELIMINATION

- 9.1 One of the most logical, and attractive, ways of reducing the incidence of gastrointestinal illness caused by VTEC (or any other food-borne pathogen) is to reduce, or even eliminate, the organism from the food chain. To do this effectively, the optimal points for intervention need to be identified (and risk assessment research can be valuable in this respect) and effective mechanisms established. Given the significance of VTEC infections, it is not surprising that there are a number of research projects in this area, with the primary focus being on reducing the spread of VTEC through the farm and into the abattoir. Alongside this is the possibility of

developing vaccines and similar approaches to protect either man or farm animals.

## 9.2 Overview of current research

### Protection of man and animals

- 9.2.1 A logical route to preventing, or at least reducing, the colonisation of cattle by VTEC is the use of some form of vaccine. A recently initiated project is looking at the possibility of developing sub-unit vaccines based on intimin and lymphostatin to reduce the carriage of EHEC in farm animals (**BBSRC LKD19295**). This approach would depend on the host animal mounting an immune response to the proteins, and thus developing immunity to the pathogen. An alternative route is to introduce appropriate antibodies to the feedstuff of the animals. Work within project **Defra OZ0703** had the aim of expressing recombinant antibodies against *E. coli* O157 in plant vectors. The early part of the project demonstrated that anti-EspA recombinant antibodies could prevent A/E lesion formation in *in vitro* models. Further *in vivo* experiments were needed to assess the potential of the approach, as well as the development of further appropriate antibodies.
- 9.2.2 Although not a solution to the reduction of VTEC infections, it would be possible to consider a form of treatment in man which involved the blocking of expression of particular genes of relevance in human disease. Using inhibitory RNA molecules targeting specific virulence factors, the possibility of turning off the pathogenic properties of the organism when in man is being investigated. One attraction of this approach is that it would not have any effect on the essential genes of the bacterium and there would therefore be no selective pressure for the micro-organism to develop resistance (**BBSRC BFP11349**). However, this line of research is no longer being pursued as alternative and preferred techniques have been found for gene silencing in *E. coli*.

### Reduction in the food supply chain

- 9.2.3 The focus of reduction and elimination of VTEC and other *E. coli* is the food chain. One possibility of an early (in the food chain) source of VTEC is contaminated animal feed (**Defra OZ0701**), and an evaluation of this source of infection is in progress (**Defra OZ0711**).
- 9.2.4 The possibility is being explored of reducing the colonisation by *E. coli* O157 of the bovine digestive tract through the inclusion in the feed of compounds which have been shown to inhibit the growth of the organism (**Defra OZ0702**). It has been shown in *in vitro* assays that plant coumarins and some other plant secondary compounds and proteins inhibited microbial growth. In parallel, the possibility that protein supplements in cattle feed might also inhibit the growth of *E. coli* was investigated, as there had been some evidence that cottonseed meal might provide

protection against proliferation and shedding of *E. coli* O157. Most supplements tested *in vitro* (including cottonseed meal) showed no antibacterial activity, but peas were shown to give reduced survival of *E. coli* O157 (**OZ0702**). The mechanism of this effect is possibly pH and further *in vivo* investigation is suggested.

- 9.2.5 A rumen-associated *Pseudomonas* strain was shown to be able to adhere to epithelial cells in culture, thus reducing the potential for *E. coli* to do the same. Further studies *in vivo* found that three out of four calves dosed with *Pseudomonas aeruginosa* showed reduced shedding of experimentally-introduced *E. coli* O157 as compared with undosed calves (**OZ0702**). Similarly, laboratory experiments are in place to investigate whether other micro-organisms can be identified which could inhibit the proliferation of enteropathogenic micro-organisms (**SEERAD RRI/504/95**).
- 9.2.6 A study to investigate the effect of various approaches to reducing the levels of VTEC O157 in farm cattle by the introduction of additional on-farm hygiene methods is now drawing to a close (**Defra OZ0138**). Other research initially studying the colonisation of the terminal rectum of cattle with VTEC, will also consider possible interventions to reduce or prevent carriage of VTEC O157 from this site (**Defra OZ0712**).
- 9.2.7 A clear picture emerges of the concern that farm and abattoir waste spread on farm land may act as a source of ongoing infection, or introduction of infection to uncolonised cattle and other farm animals (**BBSRC 0432, D12282, Defra OZ0709, WA0656, FSA B05003-4**). All five projects recommend that farm (and, where mentioned, abattoir) waste should be treated to reduce the levels of *E. coli* O157 and other pathogens before spreading. Suggested options include storage of farm waste (**OZ0709**), which was regarded as being better for solid waste than for slurries (**FSA B17002, B05003-4**). Heating of abattoir wastes and addition of calcium oxide (liming) was found to be effective in reducing *E. coli* O157 prior to application to land (**BBSRC 0432**). It was noted that the cost of these control measures was significant, and that there were potential safety issues (eg with storage of slurries) which together meant that careful attention would be needed in implementing appropriate control policies (**WA0656**). There is some research to evaluate the pollution control strategies (**SEERAD-ABRG SAC/348/03**).
- 9.2.8 Pasteurisation of carcasses is being investigated as a means of reducing the levels of *E. coli* O157 in beef (**Defra LINK AFM103**).
- 9.2.9 Addressing aspects of the food supply chain nearer to the consumer, it was noted that growth of *E. coli* O157 was almost entirely inhibited in vacuum packed meat, and was depressed when meat was anaerobically stored (**DANI 9640**). One long-term possibility for controlling levels of food-borne organisms, including VTEC, is the micro-engineering of food structure so as to reduce bacterial survival (**FSA B01001**). This proposal is based on findings that within the heterogeneous food matrix, there are microscopic "water-rich" niches in which bacterial cells can survive.

Elimination of these niches would therefore reduce bacterial survival.

- 9.2.10 The preparation of food, be it commercially or domestically, is also a significant step in which bacterial contamination can be increased or decreased. Novel routes to the commercial cleaning of raw fruit and vegetables, using ultrasound alone or in combination with chlorine, and photodynamic technology were all investigated (**FSA B02005**). The results suggested that the photodynamic process was unlikely to be more effective than the conventional chlorine treatment. With ultrasound and chlorine combined, there was a marked reduction in attached bacteria at the laboratory scale, but this did not prove to be readily scaled up to larger volumes. Research into the heat resistance of *E. coli* O157 confirmed that the ACMSF (1995) guidelines on heating of foods to reduce levels of *E. coli* O157 (Report on Verocytotoxin-Producing *Escherichia coli* (1995)) were appropriate (**FSA B02001**). High pressure treatment, as well as pH and temperature, were investigated to determine their effectiveness in terms of *E. coli* O157 inactivation. It was found that they were able to interact to increase the extent of kill of the organism in orange juice. It was noted that high pressure did not inhibit the synthesis of verocytotoxins (**DANI 9702**).
- 9.2.11 Current practice for disinfection of work surfaces in the food industry is being investigated in relation to a number of food-borne pathogens, including *E. coli* O157 (**MAFF FS3206**).
- 9.2.12 Practices in Scottish butchers' shops were found to have improved significantly since the 1996 outbreak of VTEC in Scotland, partly but not solely because of the introduction of the requirement for butchers to obtain a licence to sell both raw and cooked meat (**FSA Scotland S01011**). Independent assessors found food safety standards to be acceptable or better in 98% of supermarket butchers and 86% of independent retailers.

### 9.3 Gaps in currently funded research

- 9.3.1 In the 1999 MSFFG VTEC Report, a limited number of issues in the area of the reduction and elimination of VTEC were identified. These included:
- a need for a review of the effectiveness of carcass washing on the removal of VTEC
  - a need to review the handling of animals and carcasses so as to avoid the contamination of carcasses with VTEC: knowledge of the principal point of colonisation of cattle by VTEC may assist research in this area.
- 9.3.2 In practice, none of these has been addressed, although the handling of carcasses so as to avoid contamination with VTEC is emphasised in Defra and other guidelines (Duffy, Garvey and Sheridan (2002)).

- 9.3.3 What is clear is that the last few years have seen a major focus on the issue of transfer of pathogens through farm and abattoir wastes. Reduction of the possible contamination of food and the environment in this way is clearly desirable. However, there is still a lack of knowledge of the principal routes of transmission to man, and the frequency and geographical nature of this occurrence (see section 8, Epidemiology, above) and until more is known, it will not be easy to implement the output of further research as practical measures for reduction and elimination of VTEC in man.
- 9.3.4 For any aspects of the reduction and elimination of VTEC from the food chain, it would be important to understand how any proposed changes in practice would be received by the various components of the food supply industry, including farmers, in order to minimise resistance to the changes (**Defra OZ0144, WA0656**).

## 10. MICROBIAL ANTIBIOTIC RESISTANCE

- 10.1 There has been no research specifically investigating the resistance of VTEC to antibiotics funded by MSFFG members in the period covered by this report. Defra has, however, funded a number of strain-unspecific projects investigating antibiotic resistance of *E. coli* in relation to food-producing animals (**Defra VM02101, OZ0502**).
- 10.2 A national reference facility for antibiotic resistant bacteria isolated from food has been established (**FSA B10003**). This supports a reference service for the determination of antibiotic sensitivity of food-borne pathogens including *E. coli* O157.

## 11. SURVEILLANCE

- 11.1 In the 1999 MSFFG VTEC Report, it was recommended that the surveillance of VTEC should be addressed by the Microbiological Food Surveillance Group (now disbanded), the Epidemiology of Food-borne Infections Group and the Veterinary Surveillance Group set up under the Chief Veterinary Officer. Thus, although surveillance research would continue be listed in the MSFFG database, the group would not consider this research at its meetings.
- 11.2 There are a number of projects covered in this report which include aspects of the surveillance of VTEC (for example, see section 8, Epidemiology). In addition there is one which address specifically issues of surveillance: a database is to be set up (**FSA Scotland S01005**) to enable the gathering and analysis of all results from microbiological and chemical testing of food undertaken by local authorities.

## 12. RISK ASSESSMENT

12.1 Risk assessment and analysis is an established process in many aspects of life, including the food industry and the management of human health. It is an iterative process, which requires regular consideration of the risks associated with a particular activity or item. It is an important tool in the management of any infectious illness, and there is considerable research effort in this area, applied not only to VTEC but also to other food-borne pathogens.

## 12.2 Overview of current research

12.2.1 Risk analysis requires the development of models to identify and predict the risks of the given process. Four projects are developing models specifically to describe aspects of the risk of VTEC infection to man. Within **Defra OZ0708**, a model has been developed and used to assess the means of controlling the introduction of VTEC to abattoirs from cattle and sheep. This model led to a significant number of conclusions, addressing areas of animal husbandry and farm waste management. Most notably, any increase in the group size of cattle (or sheep) tended to lead to an increased risk of introducing VTEC to the abattoir. Sheep were less likely to cause the introduction of VTEC to the abattoir than cattle.

12.2.2 Work to develop a major risk assessment model, designed to look at the risks of introducing VTEC to humans, is seeking to consider not only the food chain but also a very wide range of environmental risks (**FSA B01019**). This project has in particular considered the differences observed in the reported incidence of VTEC O157 in Scotland as compared with England and Wales, with the intention of identifying specific factors which were associated with increased risk of infection with the pathogen. The work concluded that it was difficult to make in depth comparisons of the data as the monitoring and reporting methods in Scotland are different from those in England and Wales. However, it was recognised that there were real differences, and that the risks of infection were not only higher in Scotland, but also generally on the east side of the UK mainland.

12.2.3 The same project, which included major surveys of historical data as well as new research, concluded that the routes of transmission of VTEC to man with the highest risk were open farm visits and [use of] private water supplies (**B01019**). The latter constituted a risk as private water supplies are vulnerable to contamination with infected animal faecal material.

12.2.4 Other projects are seeking to model the behaviour and response of the bacterium, rather than the looking at transmission to the host. In **BBSRC 423124**, research is being undertaken to model and predict the response of food-borne pathogens to various environmental conditions, with VTEC as a particular focus. It is anticipated that this would provide a framework for risk assessment of VTEC. In recognition that the heating of food is a significant risk for human ingestion of VTEC, research has been

undertaken to determine the response of *E. coli* O157 to heating (to within a range of 60-80°C) (**FSA B02001**). This has confirmed that the current recommendations on heating of food to eliminate *E. coli* O157<sup>22</sup> (Report on Verocytotoxin-Producing *Escherichia coli* (1995)) provide a more than adequate safety margin.

- 12.2.5 The risks associated with the application of animal manure, farm slurries and abattoir waste onto agricultural land have been assessed (**FSA B17002**). This work has included *E. coli* O157 amongst a number of other pathogens, and has found that storage of farm yard manures significantly reduces the levels of pathogens through the action of heat, but is less effective for slurries, which are liquid based. Predictive modelling within this project enabled assessment of the effectiveness of different manure management strategies in terms of pathogen reduction. However, the research identified that further knowledge about the decay of bacteria under the various environmental conditions was needed in order to improve and strengthen this risk assessment.
- 12.2.6 The risks associated with a completely different form of storage, that of pathogen survival in foods, was the subject of research in project **FSA B01001**. One aspect of the research considered the survival, resuscitation and growth of *E. coli* and concluded that food shelf-life assessments should be based on the shortest lag times measured for the prospective pathogens, rather than the average times as is current practice.
- 12.2.7 The handling and consumption of contaminated food products is a risk factor. Research found that the prevalence of contaminated meat products at the point of consumption was low, with the prevalence of contaminated products being lowest for pork (both retail cuts and sausages) and slightly higher for sheep and beef products (**B01019**). The levels of contamination of milk from dairy cows was found to be very low.
- 12.2.8 The handling of raw meat is seen as a major point of risk for contamination of food with pathogens. It was demonstrated that bacteria could be transferred readily from the surface of raw meat to work-surfaces (**FSA B02016**). Research on raw meat handling in the kitchen has shown that *E. coli* O157 could persist on work-surfaces for at least 48 hours.
- 12.2.9
- 12.2.10 A risk assessment for VTEC O157 in milk is being developed (**Defra VF0201**).
- 12.2.11 Shellfish are often regarded as having a high risk of contamination with faecally derived bacteria. In the context of assessing methods for detecting pathogens in shellfish, no contamination by *E. coli* O157 was found in 236 samples of UK shellfish (**FSA B04008**).

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<sup>22</sup> These recommendations are to heat food at 60°C for 45 minutes, or 65°C for 10 minutes or 70°C for 2 minutes.

### 12.3 Gaps in currently funded research

12.3.1 In the 1999 MSFFG VTEC Report, it was noted that useful risk analysis could only be carried out where there were appropriate data and evidence. This is still the case, and it would appear that there is a need for significantly more data to enable the development of consistent, clear risk assessments which will be used by the farming and food production industry. It may be that the absence of substantial epidemiological data sets is an issue and that until these are developed, it will continue to be difficult for this area of work to progress.

12.3.2 The 1999 MSFFG VTEC Report identified a number of gaps in specific aspects of risk assessment. These were the need for assessment of the risk of VTEC to humans from the food chain (as opposed to other sources such as the environment or person-to-person contact); the benefit of knowing how consumers perceived the risks of VTEC from different sources and how to communicate with consumers about these; and an investigation of the effectiveness of HACCP as a risk management tool. These have generally been addressed through **B01019**. However, the current report suggests a number of areas where further research is needed, which include further quantification of levels and fate of VTEC in different environments, including animal faeces. Some aspects of this will be addressed through **Defra VF0201**.

### 13. OTHER

13.1 Although it was an expectation of the 1999 MSFFG VTEC Report that there might be research projects addressing subjects such as the socio-economic costs of food poisoning and the impact of food poisoning on society, there have been no projects in this area supported by the MSFFG during the last four years.

## 14. CONCLUSIONS

- 14.1 The research addressed in this report represents a major effort to understand the biology and control of VTEC O157, and makes a significant contribution to the international effort with this organism, the breadth of which was evident at the Edinburgh VTEC conference held in 2003<sup>23</sup>. In the past four years, UK research has made significant advances in terms of understanding the colonisation of cattle by VTEC O157 and the transfer of the organism within animals and to the food chain. There has also been advancement of the general understanding of the molecular biology of the organism, in particular linking genes to gene function and then to the host:bacterium relationship.
- 14.2 One possible issue in VTEC research is that of duplication: many researchers wish to be involved in studies of the molecular biology of *E. coli* O157 and related *E. coli*, and there is some evidence of repetitive scientific activities where, possibly, collaboration might have led to a more efficient use of resources. However, it is important that funding is not restricted to enabling only one research group to address a particular area: it is in the development of resources and agreement as to the best technologies where collaboration could be encouraged.
- 14.3 There remain significant areas where there has been less progress, and some issues identified in the 1999 MSFFG VTEC Report have not been addressed. In addition, non-O157 serotypes and strains of *E. coli*, and VTEC in particular, are being found to be associated with disease and there has been little progress in understanding these other strains.
- 14.4 Although the transmission of VTEC through the food supply chain is well researched, there is far less understanding of the relationship of VTEC with the human host. Research into the host: pathogen interaction, the immunological response of man and the epidemiology of VTEC O157 and other VTEC serotypes and *E. coli* strains in man would be valuable. Although human illness caused by VTEC is relatively rare (unlike with *Salmonella* or *Campylobacter*) and therefore the opportunity to study the epidemiology in man is limited, it is important that this area of research is pursued.
- 14.5 Exciting progress in understanding of the biology of VTEC in the laboratory also needs to be related to *in vivo* situations and to the public health issues that are a necessary driver behind VTEC research. It is an observation of this report that, with a few exceptions (eg **Defra VF0101**, **VF0201**, **VF0304**) there is not a strong link between these aspects of research. One possible reason for this is the inadequate methods for the quantification of VTEC and other *E. coli* strains *in vivo*, which makes

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<sup>23</sup> VTEC 2003, the 5th International Symposium on Shiga Toxin (Verocytotoxin) Producing *Escherichia coli* Infections

surveillance and monitoring particularly difficult.

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## APPENDIX 1: GLOSSARY

### **Abiotic surfaces**

Non-biological surfaces, such as glass, polystyrene.

### **Adhesin**

A bacterial cell surface protein that mediates attachment to mammalian cell surfaces to initiate an infection.

### **AFLP**

Amplified Fragment Length Polymorphisms. Molecular markers typical of a strain of an organism obtained by combining RFLP and PCR techniques and applying these to restriction fragments obtained from a digest of an organism's total genomic DNA.

### **AEEC**

Attaching and effacing *E. coli*

### **Attaching and Effacing lesions (A/E)**

A/E lesions are tight attachments of *E. coli* O157 and other EHEC to the gut cell wall which destroy the microvilli on the cell surface. They are essential in the pathogenicity of EHEC infections and the genes involved in the A/E lesion formation are found in the LEE pathogenicity island.

### **Bacterial interspersed mosaic elements (BIMEs)**

Small, highly repetitive DNA sequences found throughout the *E. coli* genome.

### **Bacteriophage**

Virus that infects a bacterium.

### **Coumarin**

Substance found in plants, in particular clover, with the scent of new-mown hay. Used in perfumery and to make an anti-coagulant, dicoumarin.

### **Curli**

Amyloid fibres produced by certain strains of *E. coli*, which form a meshwork around the bacteria joining them together in clusters (biofilms) which are more resistant to antibiotics and the body's immune defences. The amyloid fibres are similar to those which accumulate in the brain to form senile plaques (as in Alzheimer's disease).

### **eaeA**

A gene associated with the production of the protein intimin which mediates binding of the bacterium to the host cell surface.

### **EHEC**

Enterohaemorrhagic *E. coli*.

### **EPEC**

Enteropathogenic *E. coli*.

**espA**

A protein secreted by various strains of pathogenic *E. coli*, required by the bacterium as part of the process of attachment to host cell surfaces.

**ETEC**

Enterotoxigenic *E. coli*.

**Fimbriae**

Filaments, smaller than flagella but with a similar structure, fringing certain bacteria including *E. coli*.

**Fluorescent Labelled Amplified Fragment Length Polymorphism (fAFLP, FALP)**

See AFLP above.

**Glucuronidase-negative *E. coli***

*E. coli* O157 are generally glucuronidase-negative and so the absence of this enzyme is regarded as diagnostic for the presence of *E. coli* O157

**HACCP**

Hazard Analysis and Critical Control Point (analysis)

**Haemorrhagic colitis**

Can arise from VTEC infection and has symptoms of inflammation and bleeding from the large intestine.

**HEp-2 CELLS**

A human epithelial cell line of intestinal origin useful in the study of bacterial attachment and invasion.

**HUS**

Haemolytic Uraemic Syndrome. A clinical condition which sometimes arises from VTEC infection and is characterised by anaemia and kidney failure.

**Intimin**

A protein required by the bacterium to mediate intimate attachment to the host cell surface. Intimin cannot produce attaching and effacing lesions by itself.

**LEE**

Locus for Enterocyte Effacement. A cluster of genes located on the *E. coli* O157 chromosome, the locus encodes five secreted proteins, including intimin, which are associated with the characteristic attaching and effacing lesions of O157 VTEC infection.

**LPS**

Lipopolysaccharide. An antigenic component of the outer membrane of Gram-negative bacteria and forms part of the 'O' side chain.

**O157 VTEC**

Verocytotoxin-producing *Escherichia coli* of serogroup O157.

**PCR**

The Polymerase Chain Reaction. A widely-used technique to generate multiple copies of a target DNA sequence by amplification.

**PFGE**

Pulsed Field Gel Electrophoresis. This technique separates DNA molecules by subjecting them to alternately pulsed, perpendicularly placed electrical fields.

**Phage**

See bacteriophage.

**RFLP**

Restriction Fragment Length Polymorphisms. A technique used to distinguish between subtypes of bacteria on the basis of differences in DNA sequences and thus the size and number of restriction fragments generated.

**RT-PCR**

Reverse Transcriptase Polymerase Chain Reaction. Involves the amplification of an mRNA target sequence into a complementary DNA strand.

**Sandwich ELISA**

Enzyme-Linked Immunosorbant Assay. A technique which uses enzyme reactions as indicators. The sandwich assay is a double-layer procedure and visualises specific antibody. The antigen is sandwiched between the antibody and the secondary labelled antibody.

**SDS-PAGE**

Sodium dodecyl sulphate polyacrylamide gel electrophoresis. A method used to separate proteins according to molecular weight by adding the ionic detergent SDS such that proteins fold in a random configuration.

**STM**

Signature Tagged Mutagenesis provides a means of identifying virulence genes in bacteria which are essential for the process of infection in a chosen animal model.  
<http://www.microscience.com/stm.pdf> .

**Type III secretion system**

A specialised secretion system found in many Gram-negative bacterial pathogens, which is utilized to deliver virulence effector proteins directly into host cells.

**VTEC**

Verocytotoxin-producing *Escherichia coli* that characteristically produce powerful toxins that kill a variety of cell types, including Vero cells on which their effects were first demonstrated.

## Appendix 2: Projects from the MSFFG database used in this report

ProjectCode	ProjectTitle	Funder	StartDate	EndDate
0432	Verification and optimisation of pathogen inactivation	BBSRC	01/04/99	31/03/01
0455	Systems analysis for risk assessment	BBSRC	01/04/01	31/03/04
0754	Characterisation of the interaction of enterohaemorrhagic <i>E. coli</i> with intestinal mucosae	BBSRC	01/02/99	31/01/02
1028	Development of novel vaccination strategies for the control of enterohaemorrhagic <i>Escherichia coli</i> in cattle	BBSRC	01/06/03	31/05/04
3258371	Microbial adaptation to environment and survival in the food chain	BBSRC	01/04/98	31/03/01
4231214	Quantitative Risk Assessment for Microbiological Food Safety	BBSRC	01/04/00	31/03/03
4321208	Molecular Microbiology of <i>Salmonella typhimurium</i> and <i>E. coli</i>	BBSRC	01/04/00	31/03/03
4341058	Behaviour and prediction of microbial pathogens in food	BBSRC	01/04/99	31/03/00
841	Pathogenesis, host response and epidemiology of attaching/effacing <i>Escherichia coli</i> (AEEC)	BBSRC	01/11/00	31/10/03
B17144	Visualisation of secreted translocator and effector proteins during EPEC and EHEC O157:H7 type III secretion	BBSRC	01/09/02	01/09/05
BFP11329	Structural studies of host-cell/pathogen adhesion in enteric bacterial infection	BBSRC	01/06/99	01/06/02
BFP11335	<i>E.coli</i> diversity in ruminants and its effect on the carriage of enterohaemorrhagic <i>E.coli</i>	BBSRC	01/03/99	01/03/02
BFP11345	The role of bacteriophage in maintenance and transfer of VTgenes in gastrointestinal <i>Escherichia coli</i> populations	BBSRC	01/04/99	01/04/02
BFP11349	Inhibition of virulence gene expression by targeted redirection of RNase P	BBSRC	01/04/99	01/04/02
D10261	Characterisation of the interaction of enterohaemorrhagic <i>Escherichia coli</i> with intestinal mucosae (Joint with D10262)	BBSRC	01/03/99	01/03/02
D10262	Characterisation of the interaction of enterohaemorrhagic <i>Escherichia coli</i> with intestinal mucosae (Joint with D10261)	BBSRC	01/10/98	01/10/01
D12282	Persistence and movement of verotoxin producing <i>Escherichia coli</i> O157:H7 in soil	BBSRC	01/07/00	01/11/03
D13414	High-throughput whole-genome PCR with MADGE and SP-PCR for the analysis of genomic diversity in the enteric bacteria (joint with D 13422)	BBSRC	14/11/00	07/02/04
D13422	High-throughput whole-genome PCR with MADGE and SP-PCR for the analysis of genome diversity in the enteric bacteria (joint with 13421)	BBSRC	13/11/00	13/11/02
D13600	<i>In vitro</i> studies of the interaction between enterohaemorrhagic <i>Escherichia coli</i> and human intestinal mucosa	BBSRC	03/04/01	03/04/04
D14955	Epidemiological status and role in virulence of Antigen 43; the major autotransporter and surface antigen of <i>Escherichia coli</i>	BBSRC	05/08/02	05/08/05

ProjectCode	ProjectTitle	Funder	StartDate	EndDate
D15913	Characterisation of novel adhesin gene clusters in enterohaemorrhagic <i>Escherichia coli</i> O157	BBSRC	11/02/02	11/02/05
D17455	Role of lymphostatin in the colonisation of cattle by enterohaemorrhagic <i>Escherichia coli</i>	BBSRC	21/10/02	21/10/05
D19613	The molecular basis to <i>Escherichia coli</i> O157:H7 colonisation of the terminal rectum in cattle	BBSRC	19/05/03	19/05/06
EGA16107	Global expression in <i>Escherichia coli</i> : exploitation of genomic data to dissect a model foodborne pathogen	BBSRC	01/02/02	01/02/07
G11549	Functional analysis of highly conserved unidentified reading frames in the <i>E. coli</i> genome	BBSRC	01/11/99	01/11/02
G12163	Functional analysis of highly conserved unidentified reading frames in the <i>E. coli</i> genome	BBSRC	01/08/99	01/08/02
LKD19295	Development of novel vaccination strategies for the control of enterohaemorrhagic <i>Escherichia coli</i> in cattle	BBSRC	01/06/03	01/06/05
P10995	Cross-regulation between adhesin gene clusters in <i>Escherichia coli</i>	BBSRC	03/05/99	03/06/02
9640	An investigation of the phenomenon of cross-protection in verocytotoxigenic <i>Escherichia coli</i> O157:H7	DANI	01/05/97	01/05/00
9702	Effects of high pressure on the microbiological quality of foods	DANI	01/11/97	01/11/00
9723	Microbial quality of beef carcasses in Northern Ireland abattoirs - A baseline study	DANI	01/04/97	31/03/00
OZ0133	<i>Escherichia coli</i> O157:H7 Colonisation and Persistence in Cattle	Defra	01/04/97	31/03/00
OZ0138	A longitudinal study of faecal excretion of VTEC O157 in cattle	Defra	01/10/98	31/12/04
OZ0144	Constraints to uptake of adequate biosecurity on UK cattle and sheep farms, with special reference to zoonotic diseases.	Defra	01/06/02	30/11/03
OZ0502	<i>In vivo</i> models to investigate the development of antibiotic resistance	Defra	01/04/00	31/03/02
OZ0701	An Assessment of the Risks to the U.K Livestock Industry	Defra	01/10/98	31/03/99
OZ0702	from Animal Feed and Ingredients Contaminated with <i>E.coli</i> and other VTEC strains. Control of <i>Escherichia coli</i> O157:H7 in the ruminant gut and in the farm environment	Defra	01/04/99	30/09/02
OZ0703	Plant antibody delivery of passive immunisation against <i>E. coli</i> O157:H7; a novel means of control in the animal	Defra	01/08/99	30/06/03
OZ0704	Quantification of <i>E. coli</i> O157:H7 virulence factors <i>in vivo</i> using real-time RT-PCR and <i>in situ</i> RT-PCR	Defra	01/06/99	30/09/03
OZ0705	A proteomic approach to identify virulence determinants of EHEC O157	Defra	13/09/99	12/09/02
OZ0706	EHEC O157 Pathogenesis: Ovine and Animal Model Studies	Defra	01/04/99	31/03/04
OZ0707	Identification of Factors Mediating Colonisation of Cattle by Enterohaemorrhagic <i>Escherichia coli</i> .	Defra	01/10/99	14/03/06
OZ0708	A Systems Analysis Methodology to Elucidate and Evaluate the Critical Control Points for	Defra	01/01/00	30/06/02

## MSFFG 2004 VTEC Report

ProjectCode	ProjectTitle	Funder	StartDate	EndDate
OZ0709	<i>E. coli</i> O157:H7 in Cattle and sheep from Farm to Abattoir Epidemiology of VTEC O157 and other VTECs likely to be pathogenic to man in farm wastes	Defra	01/04/02	31/03/05
OZ0710	The role of goats and pigs in the maintenance and transmission of VTEC including EHEC O157:H7	Defra	01/10/02	30/09/05
OZ0711	Incidence and control of VTEC in animal feeds	Defra	01/08/02	31/10/05
OZ0712	<i>Escherichia coli</i> O157 interventions and control.	Defra	01/04/03	31/03/06
VF0101	Defra Cambridge Veterinary Fellowship in Microbiology	Defra	01/10/99	30/09/04
VF0201	Defra Liverpool Veterinary Research Fellowship in Epidemiology	Defra	01/09/99	31/08/04
VF0304	Defra Edinburgh Veterinary Fellowship in Pathology	Defra	01/10/99	30/09/04
VM02101	Assessment of factors influencing the development of resistance to fluoroquinolone antibiotics used in pigs and poultry	Defra	01/07/00	30/09/03
WA0656	implications of potential measures to control pathogens associated with livestock manure management	Defra	01/11/00	30/10/01
WA0804	Routes by which pathogens associated with livestock slurries and manures may be transferred from farm to the wider environment	Defra	01/09/01	30/11/04
AFM103	Steam surface pasteurisation of beef carcasses - A control point for <i>E. coli</i> O157:H7	Defra LINK	18/06/99	17/06/00
DH241	The Public Health Laboratory Service case-control study of <i>Escherichia coli</i> O157 infection in England	DH	01/07/1996	31/12/1997
DH247	A case-control study of sporadic cases of <i>Escherichia coli</i> O157 infection in Scotland 1996 to 1999	DH	01/03/1996	31/03/1999
DH254	Verocytotoxigenic <i>Escherichia coli</i> including serogroup O157: the population burden and the role of zoonotic spread.	DH	01/04/1996	30/06/1998
S01005	National Food Surveillance System For Scotland	FSA Scotland	01/03/01	31/03/04
S01011	Evaluation of butchers' licensing initiative in Scotland	FSA Scotland	01/02/02	31/07/02
S01014	Prevalence of faecal shedding on Scottish beef cattle farms of verocytotoxigenic <i>Escherichia coli</i> serotypes: O26, O103, O111 and O145.	FSA Scotland	03/01/02	30/06/04
S01018	Quantifying the seasonality of <i>E. coli</i> O157 shedding (concentration and prevalence) in cattle and estimating its effect on the number of cases of food poisoning.	FSA Scotland	13/01/02	13/04/02
B01001	Physiological and microstructural factors controlling the survival and lag of foodborne pathogens	FSA- MSD	01/04/97	31/08/00
B01007	Development & study of tests to differentiate between tolerant & sensitive isolates of <i>Salmonella</i> and <i>Escherichia coli</i> O157.	FSA- MSD	01/07/98	30/06/01
B01014	Develop a novel molecular typing method for comparison of foodborne pathogens using	FSA- MSD	01/05/99	30/04/01

## MSFFG 2004 VTEC Report

ProjectCode	ProjectTitle	Funder	StartDate	EndDate
B01019	VTEC as a model organism Development of a risk assessment model for the different pathways of infection of VTEC 0157	FSA- MSD	01/04/01	31/03/04
B02001	Thermal Death of pathogenic microorganisms in real foods	FSA- MSD	01/05/97	30/04/00
B02005	Novel Techniques for cleaning and decontaminating raw vegetables and fruit	FSA- MSD	01/04/98	01/04/00
B02016	Microbiological risk factors associated with the domestic handling of meat	FSA- MSD	01/11/00	31/10/02
B04008	Application and validation of techniques for the detection of pathogens in shellfish	FSA- MSD	01/08/00	30/11/02
B05003	Pathogens in organic wastes: their levels and survival both during storage and following application to agricultural land	FSA- MSD	01/07/99	01/02/03
B05008	The levels of pathogens in abattoir wastes	FSA- MSD	01/11/99	28/02/02
B09005	Review of microbiological methods in the food industry	FSA- MSD	01/06/98	31/03/00
B09007	Develop and validate methods to detect & characterise verocytotoxin producing <i>Escherichia coli</i> in foods	FSA- MSD	01/09/99	31/08/02
B09008	Accelerated detection of Salmonella and verocytotoxins <i>E. coli</i> in food	FSA- MSD	01/06/99	30/06/02
B09010	Develop and validate an immunological method for the detection & characterisation of all VTEC in foodstuffs	FSA- MSD	01/02/99	31/08/02
B10003	Establishment of a national reference facility for antibiotic resistant bacteria from foods; development of a national archiving facility	FSA- MSD	01/04/01	31/03/03
B11003	Diagnostic application of monoclonal antibody-based sandwich ELISA(s) for the rapid detection of Verocytotoxin-Producing <i>E. coli</i>	FSA- MSD	01/08/00	31/10/03
B11004	Development and evaluation of solid media to identify VTEC and to detect verocytotoxin in clinical and food samples by a novel method producing a different colour response to both the toxin antigen and its biological activity	FSA- MSD	10/06/96	10/06/99
B11005	Verocytotoxin producing <i>E. coli</i> : Characterisations of adhesins	FSA- MSD	01/03/96	31/5/99
B11006	Identification of novel adhesins of VTEC strains lacking the EAE determinant	FSA- MSD	01/01/97	01/01/00
B11007	Identification of novel adhesins of VTEC strains including the EAE determinant	FSA- MSD	01/01/96	01/01/00
B11008	The serodiagnosis of infections caused by Verocytotoxin-producing <i>E. coli</i> based on salivary antibodies to LPS	FSA- MSD	01/06/01	30/09/03
B14004	Generation of an archive of extracted nucleic acid for the IID archived faecal specimens	FSA- MSD	01/01/03	31/12/07
B17002	Assessment of the risks to food safety associated with spreading of animal manure and abattoir waste on agricultural land	FSA- MSD	01/05/01	30/04/02
M01011	A Study of Factors affecting MHS scores of sheep arriving at abattoirs and bacterial contamination of their carcasses	FSA MSSD	01/01/00	31/12/00
M01017	Standardisation of sampling and analysis in poultry abattoirs in support of HACCP-based	FSA MSSD	01/10/01	30/09/04

ProjectCode	ProjectTitle	Funder	StartDate	EndDate
	hygiene solutions.			
FS0035	Epidemiology and risk factor analysis of <i>E.coli</i> O157 in farm animals	MAFF	01/04/97	01/04/00
FS3206	Efficacy testing of disinfectants used in the food industry against a range of pathogens including <i>E. coli</i> O157	MAFF	01/05/98	01/11/99
BSS/028/99	The application of statistics, bioinformatics and mathematical modelling techniques to improve the understanding of the epidemiology of <i>E. coli</i> O157 infection in cattle and its transmission to humans.	SEERAD	01/10/99	30/09/04
k/mrs/50/c2601	Epidemiology and Laboratory Studies of <i>Escherichia coli</i> O157	SEERAD	01/06/97	30/11/99
k/mrs/50/c2624	Gut mucosal immunity and <i>E coli</i> O157	SEERAD	01/01/98	31/12/99
k/mrs/50/c2671	Clinical and Laboratory Follow-up of sufferers from central Scotland <i>E. coli</i> O157 outbreak	SEERAD	01/08/98	31/07/00
RRI/504/95	Antagonistic interactions between gut micro-organisms	SEERAD	01/04/95	31/03/00
SAC/125/96	Inter-relationships between livestock condition and the microbiological safety of carcasses	SEERAD	01/04/96	31/10/99
SAC/138/96	Prevalence of <i>E.coli</i> O157:H7 in Scottish livestock	SEERAD		
SAC/168/99	Determination of the prevalence of <i>E. coli</i> O157: H7 in Scottish beef cattle	SEERAD	01/01/98	31/08/00
SAC/204/98	Survival of <i>E. coli</i> O157 in Scottish soil and private water supplies	SEERAD	14/01/99	13/01/02
SAC/348/03	Implementation and evaluation of mitigation strategies for pollution control at a farm scale (PEPFAA into Practice).	SEERAD	01/04/03	31/03/06
UAB/005/97	Improved techniques for the rapid detection of <i>E. coli</i> O157 in foods	SEERAD	01/01/98	31/08/00
UAB/006/97	Characterisation of commensal and pathogenic <i>E. coli</i> populations in cattle	SEERAD-ABRG		
UAB/007/99	Survival and dispersal of <i>E. coli</i> O157 in Scottish agricultural soils, and potential for the contamination of private water supplies	SEERAD-ABRG	01/04/99	31/03/02
URG/001/96	Solid phase rapid detection of viable micro-organisms using nucleic acid amplification and biosensing techniques	SEERAD	01/04/96	30/09/99