

# **Publicly Funded Research Relating to Enterovirulent *Escherichia coli***

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- (i) verocytotoxin producing *E. coli* projects (VTEC) which  
commenced between 2004-2008**
- (ii) other enterovirulent *E. coli* projects which commenced between  
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**Report of the Microbiological Safety of Food  
Funders Group (MSFFG)**

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### **SUMMARY**

This report provides an update on the research funded by the member organisations of the MSFFG on enterovirulent *Escherichia coli*. Projects which are included are: i) those describing research on verocytotoxin-producing *E. coli* (VTEC) which commenced over the period 2004-2008 and ii) projects describing research into other enterovirulent *E. coli* which started during 2000-2008.

The report describes a total of 85 projects which fall broadly into two categories; those investigating the basic fundamental research into bacterial physiology and pathogenicity, and those with an applied research focus. Many of the fundamental research projects have a molecular biology element which reflects the availability of *E. coli* genome sequences, while the applied projects focus largely on VTEC and the detection, reduction and elimination of the organism from cattle and the food supply chain.

Enterovirulent strains of *E. coli* cause gastrointestinal diseases in humans. The type of enterovirulent disease caused by a particular strain of *E. coli* depends on the distribution and expression of virulence factors. Several clinical syndromes accompany infection with enterovirulent *E. coli* and include different types and duration of diarrhoea (e.g. persistent, watery) and also a range of side effects including vomiting, pyrexia and abdominal cramps. VTEC infections are rarer, but can lead to haemorrhagic colitis and/or haemolytic-uremic syndrome which can be fatal. VTEC is also sometimes known as enterohaemorrhagic *E. coli* (EHEC) or shiga toxin producing *E. coli* (STEC).

Infected humans and carriers are the main source of most enterovirulent *E. coli* infections. The key reservoir for VTEC is the gastrointestinal tract in healthy farm cattle. A number of potential routes of infection arise from a cattle reservoir, including contamination at various stages of the food production chain, from the farm, to abattoir to food production and the kitchen. The environment where infected animals are maintained has also been identified as a source of VTEC infection.

#### Significant advances achieved through research

A significant number of projects have investigated the pathogenicity of *E. coli* at the molecular biology level. Greater insights into gene function, the

identification of additional putative virulence factors, and comparative genome studies have all been possible due to the availability of genome sequences.

The information gained from those projects which describe research into VTEC in the context of the human food-supply chain appears to have consolidated earlier information regarding key contamination sources and ways of reducing levels of VTEC in farm animals. Mathematical models are also described which simulate VTEC transmission. The data available from projects have been used to produce strategies to reduce the risk of contamination from the farm animal and in the food processing industry.

#### Outstanding issues

This report highlights gaps which, if addressed with additional research, would add to the current knowledge of enterovirulent *E. coli* in relation to the microbiology of food.

- There are less molecular biology data generated from *in vivo* models of the host (cattle):human (or human model) system than there are *in vitro* data sets. If such data were available it could both help validate the *in vitro* models and lead to a greater understanding of the relationship between the pathogen and host<sup>1</sup>.
- In relation to transmission and colonisation there is a need for a better understanding of the conditions needed for successful transfer of VTEC from animals to man, including the risk of VTEC infections from non-food sources and what preventative strategies, if any, are recommended.
- The risk of transfer of the virulence determinants from VTEC to other organisms was highlighted in the previous VTEC report and remains a gap.

Overall the projects described in this report provide data and information both of the fundamental biology of enterovirulent *E. coli*, and the presence and control of VTEC in the food chain in the context of human health. There remains a need to bring together both of these areas of research to ensure that information on pathogenicity can be applied to further current understanding of the relationship between host and pathogen.

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<sup>1</sup> In the 2001 Nature paper on Genome sequence of enterohaemorrhagic *Escherichia coli* O157:H7 the authors suggested that “..... showing that disease-related traits are associated with predicted genes will require many areas of study including extensive testing in animal models that mimic symptoms of human infections.....” (Perna *et al*, 2001).

## LAY SUMMARY

*Escherichia coli* are commonly known as *E. coli* and are bacteria which live in the digestive tract (the stomach and intestines) of humans and animals. While most *E. coli* are harmless, there are a number which can infect the intestines and cause vomiting and diarrhoea. There are many varieties of *E. coli*, which are assigned to one of six groups based on the range of symptoms they cause.

The most harmful group is called the verocytotoxic or verocytotoxin-producing *E. coli* (VTEC), and the most common strain in the UK is *E. coli* O157. This type of *E. coli* can cause severe illness, and in some cases complications which can cause death.

VTEC live in the intestines of healthy cattle and other animals and are present in their faeces which means they can be present on the land and in the water of the fields where the cattle live, and can potentially also be present in unpasteurised milk. The bacteria can also contaminate meat and the surrounding machinery which is used during the production of foods. Other *E. coli* that cause vomiting and diarrhoea are not thought to be linked with an animal and the main source of infection is from the faeces of other infected humans.

Humans can become infected with VTEC *E. coli* by eating contaminated undercooked meat, especially minced meat products, or from unpasteurised dairy products. Other foods such as cooked meats, sandwiches, vegetables, salads, fruit and juices can also become contaminated and have been reported as sources of VTEC outbreaks.

A second major source of VTEC infection is a faecally contaminated environment, for example farms that are open to the public, where the bacteria can be caught from direct contact with animals, their faeces or the environment. Infection can also be acquired on farms by contact with faecally-contaminated water, including that used for irrigation of crops.

The seriousness of VTEC infection has prompted research to understand how and why the bacterium causes such a severe illness, and how farming and food production practices can be improved to reduce the potential risk to the consumer.

This report summarises current UK research which has investigated *E. coli* which cause gastroenteritis. It focuses on work to develop a greater understanding of the biology of *E. coli* and in particular on VTEC in relation to food production processes.

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## INTRODUCTION

### 1. Background on enterovirulent *E. coli*

- 1.1. *Escherichia coli* are facultative bacteria of the gastrointestinal tract of humans and animals. Whilst commonly commensal bacteria, some *E. coli* strains are pathogenic as they cause disease of the gastrointestinal, urinary, or central nervous systems. This report focuses on enterovirulent *E. coli* which cause gastrointestinal tract disease in humans.
- 1.2. Gastrointestinal diseases are caused by enterovirulent strains of *E. coli* which can be divided into categories each with distinct pathogenic schemes. The type of enterovirulent disease caused by a particular strain of *E. coli* depends on the distribution and expression of virulence factors, for example, adhesin molecules and toxins. Several clinical syndromes accompany infection with enterovirulent *E. coli* and include different types and duration of diarrhoea (e.g. persistent, watery) and also a range of side effects including vomiting, pyrexia and abdominal cramps.
- 1.3. Over 700 antigenic types (**serovars**) of *E. coli* are recognized based on **O (somatic)**, **H (flagellar)**, and **K (capsular)** surface antigen profiles (Todar (2008)). At one time serotyping was important in distinguishing the strains that actually cause disease. More recently, particularly for enterovirulent strains, identification and classification of the pathogenic *E. coli* has been based on their virulence factors.
- 1.4. For the purpose of this report, the term enterovirulent *E. coli* refers to six classes of enterovirulent *E. coli* listed below (Riemann and Cliver (2006), Labbe and Garcia (2001)). The term VTEC refers to all verocytotoxin-producing *E. coli*. There are a number of serovars of VTEC which have particular relevance to humans, for example O157:H7, O145, O111, O26 and O103.

**enterohaemorrhagic *E. coli* (EHEC)** This strain is also described as **verocytotoxic** or **verocytotoxin-producing *E. coli* (VTEC)**, or **shiga toxin producing *E. coli* (STEC)**.

**enterotoxigenic *E. coli* (ETEC)**  
**enteropathogenic *E. coli* (EPEC)**  
**enteroaggregative *E. coli* (EAEC)**  
**enteroinvasive *E. coli* (EIEC)**  
**diffusely adherent *E. coli* (DAEC)**

- 1.5. A further class of enterovirulent *E. coli* is **adherent invasive *E. coli* (AIEC)** which has been found to be associated with the inflammatory bowel diseases, Crohn's disease and ulcerative colitis (Rolhion and Darfeuille-Michaud (2007)).

- 1.6. The most common strain of VTEC detected in the UK in human infections is *E. coli* O157:H7. Other serovars are less commonly observed or investigated and are therefore harder to diagnose. *E. coli* O157:H7 was first reported as a cause of gastrointestinal illness in humans in 1982 (Riley *et al.*, (1983)), and this serovar has the greatest impact on the human population in those countries with high sanitation standards. VTEC pose a significant hazard. The infectious dose for VTEC O157:H7 is estimated to be only 10 - 500 cells<sup>2</sup> which is a much lower level than figures reported for other enterovirulent *E. coli*, for example ETEC (10<sup>6</sup> - 10<sup>8</sup>), EPEC (10<sup>6</sup>) and EIEC (10<sup>6</sup>) (Labbe and Garcia (2001)). Unlike the other enterovirulent *E. coli*, VTEC, especially O157:H7, causes the particularly serious clinical conditions of Haemorrhagic Colitis (HC) in around 50% of cases<sup>3</sup> and Haemolytic Uraemic Syndrome (HUS) in around 5% of cases. Thrombotic Thrombocytopenic Purpura (TTP) can also occur and can have a mortality rate in the elderly as high as 50%<sup>4</sup>. A percentage of those developing these more serious infections go on to develop long-term health problems such as kidney disease.
- 1.7. The combination of a relatively low infectious dose and the serious morbidity and mortality provides a basis for the focus of projects on *E. coli* O157:H7. A further important factor is the animal reservoir, and the relationship of some infections to food consumption for this type of *E. coli*.

## 2. The importance of the microbiological safety of food in relation to VTEC

- 2.1. The reservoirs for the classes of enterovirulent *E. coli* vary and this has an impact on the relationship of the organism to contaminated food. The main reservoir for VTEC is the gastrointestinal tract in healthy cattle. Although *E. coli* O157:H7 has also been isolated from the intestinal tract of sheep, deer and other animals (Riemann and Cliver (2006)), cattle are the significant reservoir for the transmission and maintenance of this strain. Unlike VTEC, other enterovirulent *E. coli* do not have a clear association with an animal reservoir, hence infected humans and carriers are considered to be the main source of infection by ETEC, EPEC, EIEC, EAEC and DAEC (Labbe and Garcia (2001), Riemann and Cliver (2006)).
- 2.2. Transmission of enterovirulent *E. coli* occurs via the faecal-oral route, hence most outbreaks result from exposure of humans to contaminated food/water, contaminated environment (e.g. farms which are open to the general public) or person to person transmission.
- 2.3. The risk of transmission for ETEC, EPEC, EIEC, EAEC and DAEC is low in countries having high sanitary standards and practices, but is an important cause of diarrhoea in infants and travellers in underdeveloped countries or

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<sup>2</sup> <http://www.foodsafety.gov/~mow/intro.html>

<sup>3</sup> HPA 2007 report <http://www.hpa.org.uk/cdph/issues/CDPHvol3/No1/vtec.pdf>

<sup>4</sup> <http://www.foodsafety.gov/~mow/intro.html>



regions of poor sanitation. Food becomes contaminated from infected humans via poor sanitation, and food handling or processing.

- 2.4. Unlike the other enterovirulent *E. coli* where contamination is from the human host, the colonisation of VTEC in the intestines of farm animals leads to a number of potential routes of infection. The organism can enter the food chain by i) faecal contamination of raw meat directly from the animal host during the process of evisceration and fleece/hide removal, ii) faecal contamination of meat from faeces present on slaughterhouse equipment or within the slaughterhouse environment and, less commonly, iii) by faecal contamination of unpasteurised milk on the farm. A second important source of VTEC O157 of animal origin is a contaminated environment, for example farms which are open to the public, where transmission to man occurs via direct contact with animals, their faeces or through the environment. Infection can also be acquired through consumption or contact with faecally-contaminated water, including that used for irrigation of crops.
- 2.5. VTEC O157 is killed by adequate cooking of meat and pasteurisation of milk, but consumption of undercooked meat and raw dairy products has resulted in outbreaks of infection. Cross-contamination of ready-to-eat foods such as cooked meats can occur with reports of outbreaks linked to vegetables, salads, fruit and unpasteurised juices<sup>5</sup>.

### **3. Epidemiology in the UK**

- 3.1. In 2008 there were 1248 laboratory confirmed cases of VTEC O157 infection reported in the UK (England and Wales (948), Scotland (241), Northern Ireland (59)), an increase on the numbers in 2007 (1113), but at a similar level to that seen in 2006 with 1234 cases.
- 3.2. Figure 1 shows that there are differences in the geographical distribution between countries. Scotland showing a consistently higher prevalence of approximately 4 cases per 100,000, as opposed to approximately 2 cases per 100,000 in England and Wales, and Northern Ireland.
- 3.3. The largest reported outbreak of *E. coli* O157 occurred in Osaka, Japan in July 1996 where there were 2,764 microbiologically confirmed cases of which 2,345 were school children from 47 schools. There were 121 cases of HUS resulting in three deaths. The source of the outbreak was identified as radish sprouts being served at schools (Pennington (2009)).

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<sup>5</sup> <http://www.cfsan.fda.gov/~mow/chap15.html>

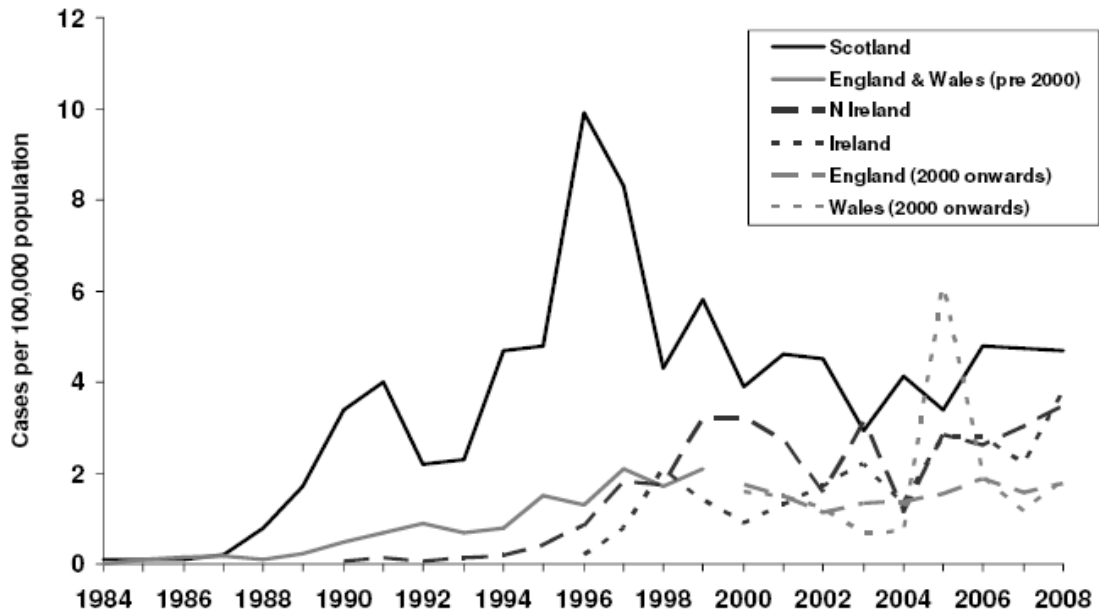


Figure 1: Isolation rates per 100,000 of the population of *E. coli* O157 confirmed in laboratory isolates from humans<sup>6</sup>

- 3.4. The largest outbreak of *E. coli* O157 in the UK occurred in central Scotland during November 1996 where there were 279 confirmed cases of *E. coli* O157 and more than 2,500 people were investigated. There were 17 deaths either caused by *E. coli* O157, or in which infection with it was a significant contributory factor (reviewed in Pennington (2009)).
- 3.5. More recently, an outbreak of *E. coli* O157 occurred in South Wales in September 2005 which was the second largest outbreak in the UK. A total of 157 cases were identified, of which 118 were confirmed microbiologically as *E. coli* O157. Of those, 109 were of a strain unique to the outbreak. Most cases were children in 44 schools across the area. Thirty-one people were admitted to hospital and one child aged five, died. A public enquiry into the outbreak was led by Professor Hugh Pennington and a full copy of the report is available<sup>7</sup>. In summary, the outbreak was caused by consumption of cooked meats that had been contaminated with *E. coli* O157 present on meat from an abattoir.
- 3.6. The risks associated with VTEC O157 are still very real and support the need for continued research in this area. A summary of cross funding body research on enterovirulent *E. coli* can help identify gaps and areas of research which will lead to a greater understanding these organisms.

<sup>6</sup> <http://www.documents.hps.scot.nhs.uk/giz/graphs/2008/rates.pdf>

<sup>7</sup> <http://wales.gov.uk/ecolidocs/3008707/reporten.pdf?lang=en>

#### 4. Previous MSFFG reports

- 4.1. VTEC projects have been the focus of previous reports from the MSFFG on *E. coli*, as VTEC causes serious illnesses with a high mortality rate especially in the context of the other enterovirulent *E. coli* and most other food-borne zoonoses.
- 4.2. Previous reports concerning VTEC have been produced by the MSFFG based on project reports in the MSFFG database. The initial report (*UK publicly-funded research relating to verocytotoxin-producing Escherichia coli VTEC*) was issued in 1999 and summarised the publicly funded research for the period 1990 to 1999 related to VTEC O157, as at the time this was the most frequent serovar isolated in the UK<sup>8</sup>. An update report was written in 2004<sup>9</sup> which had a broader remit to include research involving all verocytotoxin-producing *E. coli* (VTEC) that are pathogenic to man and of importance in food safety. In this document this report will be referred to as the 2004 VTEC report.
- 4.3. No reports on more general research projects on enterovirulent *E. coli* research have been published by the MSFFG group to date as the focus has been on VTEC.

#### 5. Focus of this report

- 5.1. Since 2004 there have been a number of new projects undertaken on VTEC O157 identified in the MSFFG database. This report provides an updated summary of research showing what progress there has been in understanding the pathogenicity of this organism and also studies into the relationship between this organism and the food chain. In addition a comprehensive review on VTEC was commissioned by the Food Standards Agency in 2004, ending in 2008, and this provides information on the state of knowledge of VTEC up to that time (**FSA B11010**).
- 5.2. In addition to research projects in which the focus of investigation is VTEC, there are a number of projects describing research into the other enterovirulent groups of *E. coli* that are included in the MSFFG database. A number of these projects describe research applicable to both VTEC and enterovirulent non-VTEC. Given that these projects remain to be described and summarised in a MSFFG report, they have been included in this report alongside the update of VTEC research.

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<sup>8</sup> <http://www.food.gov.uk/multimedia/pdfs/verocytotoxin.pdf>

<sup>9</sup> <http://www.msffg.org.uk/reports/reports/vtec2.pdf>

## 6. Research funded by other funding bodies

### Within the UK

- 6.1. Within the UK the majority of research on enterovirulent *E. coli* is funded by the BBSRC, FSA and Defra. A number of projects on *E. coli* are funded by the research councils (MRC, BBSRC, Wellcome Trust) but have been excluded from discussions here as they do not specifically include research using verocytotoxic or enterovirulent strains of *E. coli* in relation to food borne illnesses. Similarly, projects using other model organisms or systems and which may have broader relevance have not been considered.
- 6.2. Between 2003 and 2008 The Wellcome Trust funded 14 projects on enterovirulent *E. coli* and/or enteropathogenicity totalling £4.7 million<sup>10</sup>. A multidisciplinary project was also funded from 1999 to 2005. This project '*International Partnership Research Award in Veterinary Epidemiology (IPRAVE)*<sup>11</sup> : *Epidemiology and evolution of Enterobacteriaceae infections in humans and domestic animals*' focused on food-borne, zoonotic verocytotoxic *Escherichia coli* strains (especially O157) and *Salmonella* Typhimurium, and through a series of field studies, collected over 7500 isolates which were made available to the scientific community. The Medical Research Council research database<sup>12</sup> (2004-2008) records 5 grant awards which focus on enterovirulent *E. coli*, two of which describe research into EHEC and EPEC.

### Within EU funded Europe

- 6.3. Details of projects wholly or partially financed from the budget of the European Commission are held on a Research and Technological Development (RTD) database which contains information on projects from 1986 onwards, and is maintained by the Community Research and Development Information Service (CORDIS)<sup>13</sup>.
- 6.4. A total of 24 projects which described research applicable to enterovirulent strains of *E. coli* were identified in the database. Eleven of the 24 projects commenced after 2000 and two projects are current. The research topics within this group of more recent projects include: assay development for diagnostics (3 projects), prevention (1), genomic analysis/manipulation (4) and risk assessment and prevention (1).
- 6.5. An ongoing project (36256) funded by Framework Programme 6 is the Pathogenic *Escherichia* Network (PEN). This consists of a large number of international research groups (European Union, USA, Chile, New Zealand, Israel, and Australia) working on *E. coli* O157 and other potentially

<sup>10</sup> <http://www.wellcome.ac.uk/Funding/Grants-awarded/WTX022348.htm>

<sup>11</sup> <http://www.wellcome.ac.uk/Professional-resources/Biomedical-resources/Micro-organisms/WTD020697.htm>

<sup>12</sup> <http://www.mrc.ac.uk/Utilities/Documentrecord/index.htm?d=MRC002629>

<sup>13</sup> <http://cordis.europa.eu>

emerging pathogenic strains. This network is building on the previous *E. coli* Concerted Action (CT98 3935) project. In 2008 an international conference on Epidemiology and Transmission of VTEC and other Pathogenic *Escherichia coli* was held in Sweden as part of the PEN project and discussed topics such as risk factors, surveillance and molecular epidemiology.

- 6.6. A second large European Communities project funded under the International Cooperation programme was *COST action (Foodborne Zoonoses: a Co-ordinated food chain approach)*. This project involved researchers from 12 European countries and was completed in 2006. The main objective of the project was to achieve a better control of foodborne zoonotic infections by investigating new and emerging pathogens, harmonising diagnostics and typing and assessing the survival of pathogens through the food chain.
- 6.7. The programme on food and waterborne diseases (FWD) unit of the European Centre for Disease Prevention and Control (ECDC)<sup>14</sup>. The FWD has responsibility for surveillance networks that are or have been functioning at the EU level. The coordination of the former network Enter-net was transferred in 2007. In 2008, surveillance focused on six priority diseases: salmonellosis, campylobacteriosis, VTEC/STEC infection, listeriosis, shigellosis and yersiniosis.
- 6.8. In 2004 the Sixth Framework Programme (FP6A) 'Food quality and safety' theme funded the EU MED-VET-NET network which aims to unite medical and veterinary expertise in the fight against zoonoses and make better use of resources by sharing facilities internationally, centralising archives of reference material and standardising procedures to enable information to be pooled. A number of EU MED-VET-NET projects relevant to *E. coli* can be found listed on the MSFFG database (**MVN-WP04, 07 and 26**).

#### Within the US

- 6.9. The scope of the information provided here is from the National Institute of Health database of funded research<sup>15</sup>. This database includes projects funded by the National Institutes of Health (NIH), Substance Abuse and Mental Health Services (SAMHSA), Health Resources and Services Administration (HRSA), Food and Drug Administration (FDA), Centers for Disease Control and Prevention (CDCP), Agency for Health Care Research and Quality (AHRQ), and Office of Assistant Secretary of Health (OASH).
- 6.10. The database has recorded 500+ research projects on *E. coli*, with a subset of 47 projects researching aspects of the enterovirulent strains (ETEC, EPEC, EAEC, EIEC, DAEC, and VTEC). Topics investigated were not

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<sup>14</sup> [http://ecdc.europa.eu/en/activities/diseaseprogrammes/pages/programme\\_on\\_food\\_and\\_waterborne\\_diseases\\_and\\_zoonoses.aspx](http://ecdc.europa.eu/en/activities/diseaseprogrammes/pages/programme_on_food_and_waterborne_diseases_and_zoonoses.aspx)

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

dissimilar to those described in the previous VTEC 2004 report, and include the fundamental biology and genetics of pathogenicity and colonisation (36/47), prevention /reduction/treatment (7/47) and model development (4/47).

### Global initiatives

- 6.11. The World Health Organisation (WHO) has a department of Food Safety, and Zoonoses (FOS) and is developing a comprehensive strategy on strengthening food-borne disease surveillance. In September 2006, it convened an international consultation during which FOS and its multiple partners took stock of the currently available evidence and charted the strategic way forward to fill the data gap. This meeting prompted the launch of the initiative to estimate the global burden of foodborne diseases from all major causes<sup>16</sup>. The WHO has also sponsored a number of earlier initiatives including in 2001 on *Global surveillance of foodborne disease: developing a strategy and its interaction with risk analysis* and in 2002 a consultation event in Europe on *Method for foodborne surveillance in selected sites*.

## **7. Scope and structure of this report**

- 7.1. The MSFFG database<sup>17</sup> is populated with research projects relevant to the microbiological safety of food funded by the member organisations and contains project information dating back to 1992. A search of the database for projects that commenced between 1992 and 2008 using the term *E. coli* identified 346 projects. Many further projects on *E. coli* are funded by the research councils (MRC and BBSRC) but have been excluded from the MSFFG database as they do not specifically include research using verocytotoxic or enterovirulent strains of *E. coli* in relation to food borne illnesses. Similarly, research using other model organisms or systems and which may have broader relevance is not included. Post-graduate PhD studentships are also excluded from the MSFFG database.
- 7.2. In order to show the more recent progress in the understanding of the pathogenicity of enterovirulent *E. coli* the MSFFG agreed to focus on projects addressing enterovirulent *E. coli* from 2000 to 2008, after considering the science of the earlier projects either to have been superseded or included within more recent research.
- 7.3. The report also includes the summaries of VTEC projects undertaken between 2004 and 2008 to update the previous VTEC reports.
- 7.4. This report is based on 85 projects identified in the MSFFG database. Funders of the projects are listed in Table 1. Eighty eight percent of all

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<sup>16</sup> [http://www.who.int/foodsafety/publications/foodborne\\_disease/burden\\_sept06/en/index.html](http://www.who.int/foodsafety/publications/foodborne_disease/burden_sept06/en/index.html)

<sup>17</sup> [www.msffg.org.uk](http://www.msffg.org.uk)

projects are funded by the FSA, DEFRA or BBSRC, with the other MSFFG member organisations, funding two or less projects.

- 7.5. The summaries of research projects are described in detail below. The initial sections (10 and 11) summarise projects describing basic fundamental research into the biology of enterovirulent *E. coli*. This includes studies into, for example, virulence factors present in the outer membrane of the bacteria, genomic studies and colonisation strategies. The sections have been subdivided into those projects that are VTEC specific and those which are applicable to all enterovirulent *E. coli* (including VTEC).
- 7.6. The sections (12 to 16) describing the applied research projects have a much greater focus on VTEC rather than the broader group of enterovirulent *E. coli*. Where appropriate the applicability to the broader group is indicated.

MSFFG Funder	Number of projects funded
Biotechnology and Biological Sciences Research Council (BBSRC)	47
Food Standards Agency (FSA) including FSA Scotland	19
Department for Environment, Food and Rural Affairs (Defra)	9
Department of Agriculture and Rural Development (Northern Ireland) (DARD)	2
Scottish Executive Environment and Rural Affairs Department (SEERAD)	1
Medical Research Council (MRC)	2
Food Safety Promotion Board (FSPB)	1
The Environment Agency	1
Meat and Livestock Commission† (MLC)	1
Health Protection Agency (HPA)	1
Research Councils UK (RCUK)*	1

† Meat Livestock Commission resigned their membership of the MSFFG in 2007.

\* Research Councils UK (RCUK) is a strategic partnership between the seven UK Research Councils which was established in 2002

**Table 1. Allocation of enterovirulent *E. coli* projects funded by the MSFFG**

## 8. Methodology

- 8.1. The MSFFG project database was used to identify projects for inclusion in this report. A broad search of the whole database (including archived projects) was undertaken using the following terms:

*Escherichia coli*, VTEC, O157, 0157, EHEC, STEC, SLTEC, O26, O111, O103, O145

## MSFFG Enterovirulent *E. coli* report 2000-2008

A project data spreadsheet was generated by combining the databases. Any inappropriate records were removed from the list. Those projects with a project start date in 2000 onwards were identified. Projects in the spreadsheets were then marked to indicate if they had already been discussed in previous VTEC reports. This system ensured that there was no duplication and also captured any outstanding 2000-2004 VTEC reports. The list of projects is given in Appendix 2.



**MSFFG funded *Escherichia coli* research i) VTEC research 2004 – 2008 and ii) other enterovirulent *E. coli* research 2000 - 2008**

**9. Summary data**

- 9.1. A total of 85 projects have been identified in the database. The projects are grouped according to i) whether they are VTEC specific, or ii) describe research that is applicable to more than one type of enterovirulent *E. coli* (including VTEC). Projects have also been categorised as either basic or applied based on the research information provided in the database. Basic research is considered to be projects that carry out fundamental studies (often structural, biochemical, or genetics based) directed towards a better understanding of the pathogenicity of *E. coli*. Projects which describe research into solving the practical issues of enterovirulent *E. coli* as it related to the food chain or human illness are considered as being applied.
- 9.2. Of the 85 projects, 59% (50) can be described as basic research and 40% (34) as applied. An additional project, funded by the Food Standards Agency (**FSA B11010**), is a review of published VTEC research between 2000 and 2007 which includes descriptions of both basic and applied research. Fifty four per cent (46) of the projects describe VTEC specific research while 46% (39) of projects focus upon research applicable to all enterovirulent *E. coli*.

**10. The biology of enterovirulent *E. coli* and their relationship with pathogenicity and colonisation.**

- 10.1. The pathogenicity and colonisation ability of *E. coli* is associated with the expression of distinctive bacterial properties, products, or structures referred to as virulence factors. Virulence factors can be structural proteins located in the outer membrane of the bacteria, may be a protein or metabolite involved in the metabolism of the bacteria or are molecules designed to exert their effect following their export from the bacteria and subsequent insertion into the host cell. Additional protein structures and metabolites can also be present in the bacteria which function in conjunction with the virulence factors. The following sections describe projects which are investigating a range of physiological components. While a number of the projects are investigating VTEC specific mechanisms, others have a broad applicability to enterovirulent *E. coli*.

The twin-arginine translocation (Tat) complex

All enterovirulent *E. coli*

- 10.2. The Tat complex is a trans-membrane protein which is used to secrete fully folded virulence factors across the bacterial membrane in some strains of *E. coli*. A functional genomic characterisation of the Tat complex was undertaken (**BBSRC BBSEJ0000A221**), and a structural and functional analysis of virulence factors that it delivers, together with protein factors and

pathways required for the assembly of proteins onto the outer bacterial envelope were investigated (**BBSRC BBSEJ0000A189** and **P16414**).

The Type-III secretion system (TTSS) and locus for enterocyte effacement (LEE).

VTEC and EPEC

- 10.3. Verocytotoxin producing *Escherichia coli* (VTEC) and Enteropathogenic *E. coli* (EPEC) possess a type-III secretion system (TTSS) encoded for by the locus for enterocyte effacement (LEE). The TTSS translocates proteins into host intestinal cells which then subverts eukaryotic signalling pathways e.g. inflammatory responses, tight junction barrier function, the cell cycle, mitochondrial function and apoptosis and also target the cytoskeleton.
- 10.4. A number of studies were undertaken to develop a more detailed understanding of the structure and assembly of the TTSS and its associated LEE. Research was undertaken to assess the molecular process of assembly of “pedestal-like” pseudopods (part of TTSS) which bind the bacteria to the host cell surface (**MRC G0500583**), and electron microscopy combined with *in situ* immunogold labelling was used to examine the structure of the EPEC:VTEC TTSS, its assembly and the secretion of effector proteins (**BBSRC B17144**).
- 10.5. It was thought that only a small number of effectors were translocated through the TTSS in *E. coli*. However, through genomic data-mining, research has identified nearly a hundred novel effector candidates, in the *E. coli* O157 genome and investigated how many were translocated into human cells and where they localized (**BBSRC BBD0101951**).
- 10.6. Transcription of LEE genes is effected by regulators, EtrA, EivF and Ler, which exert a profound effect in EHEC (but not in EPEC). Direct targets of EtrA and EivF were identified (**BBSRC BBC5167011**) and research was undertaken (**BBSRC BBE0208601**) to study the Ler regulator.

Other bacterial components associated with pathogenicity

- 10.7. VTEC/EPEC infection is characterised by an attachment of the bacteria to the host cell via an outer membrane protein (intimin) which binds to a protein receptor in the host membrane. This receptor was originally thought to be a host protein, but has recently been found to be of bacterial origin. Enterohaemorrhagic *E. coli* has been used to identify and characterise this host cell receptor (**MRC G0401551**).

All enterovirulent *E. coli*

- 10.8. A detailed mass and atomic force spectroscopy investigation of the folding and polymerisation of chaperone: polysaccharide subunit complexes of the

F1 capsule polysaccharide is relevant to the adhesive organelles found in pathogenic *E. coli* and *Salmonella* (**BBSRC B16926**).

- 10.9. Genetic and biochemical characterisation of the Rcs two-component pathway in *E. coli* was undertaken in order to understand its regulatory role in gene expression during the formation of biofilm surface-associated communities formed following transition from planktonic growth to growth on a solid surface (**BBSRC P16371**).
- 10.10. The role of KpsD protein in the export of surface associated polysaccharides across the outer-membrane in *E. coli* was investigated by analysis of the KpsD topology, structural function and interaction with the peptidoglycan cell wall (**BBSRC BBSB09627**).
- 10.11. An autotransporter secretion mechanism which occurs in EAEC uses a plasmid-encoded toxin (pet) to produce cytotoxic and enterotoxic effects on the host. Aspects of this autotransporter secretion: inner membrane, periplasmic and outer membrane translocation were investigated (**BBSRC P14130**).
- 10.12. While not directly investigating *E. coli*, project **BBSRC BB/C509807/1** includes highly relevant research with full characterisation of tripartite ATP-independent periplasmic (TRAP) transporters. These proteins are thought to be involved in the transport of free sialic acid which is removed from the host cell surface by a virulence factor synthesised by bacterial pathogens.

### Toxins

- 10.13. Some strains of *E. coli* (VTEC, ETEC and EAEC) produce toxins which causes severe damage to intestinal epithelial cells. VTEC produces toxins which are termed either verocytotoxins (giving the acronym VTEC) or Shiga-like toxins (giving the acronym STEC, for Shiga toxin producing *E. coli*).

### VTEC

- 10.14. The inter-relationship between the expression, synthesis and release of toxin, phage mediated lysis, and the SOS response (an inducible DNA repair system that allows bacteria to survive sudden increases in DNA damage) in Shiga toxin-producing *E. coli* (STEC/VTEC) was investigated (**BBSRC D12994**).

### Metabolism of enterovirulent *E. coli* in response to environmental factors

#### All enterovirulent *E. coli*

- 10.15. Bacteria can utilise a wide range of inorganic and organic nitrogen sources and the mechanisms that regulate the genes required for this metabolism are complex. The success of *E. coli* and its ability to colonise and survive

stress conditions is likely to be related to a number of adaptive metabolic responses.

- 10.16. The flavohaemoglobin Hmp of *Escherichia coli* and *Salmonella* provides protection from nitric oxide (NO) and nitrosative stress. An evaluation of Hmp (**BBSRC P18939**) dissected the roles of the haem and flavin domains and identified additional mechanisms for NO and nitrosative stress tolerance. The genetic responses mounted by *E. coli* to nitrosative and oxidative stresses *in vitro* were also studied (**BBSRC P15901**).
- 10.17. The transporter CydDC is essential for assembly of cytochrome bd, an oxidase that is critical for growth under conditions of stress and is observed to be required for pathogenicity. The role of CydDC in the maintenance of redox homeostasis was investigated (**BBSRC BB/C514174/1**).
- 10.18. A genomic and metallomic characterisation of *E. coli* examined the effect of zinc on the expression of genes, some of which were found to be involved in antibiotic resistance (**BBSRC BBC5098311**).
- 10.19. The effect of environmental signals, or the infection process on the expression of genes involved in *S. Typhimurium* and *E. coli* infection was investigated (**BBSRC BBSEF00041208**) using a functional genomic approach with DNA microarrays, proteomics and Green Fluorescent Protein (GFP).

#### Induction of virulence in commensal *E. coli*

- 10.20. Virulence can be induced in *E. coli* as the bacteria have the ability to acquire genetic information from a number of sources including bacterial viruses or plasmids. The genetic information can become incorporated into the bacterial DNA and is thus transcribed and translated along with the bacteria's own DNA.

#### VTEC

- 10.21. Verocytotoxin-encoding bacteriophages (VT-phages) are responsible for the toxigenic conversion of *E. coli* by integration of their verocytotoxin genes into the host genome. These infection processes are thought to have important implications for pathogenicity. A VT-phage model (with a bank of VT-phages) was used in project **BBSRC BBSB05265** to characterise the mechanisms that dictated binding to the bacterial host cell following VT-phage contact, phage immunity and integration into the host genome.

Other factors that affect successful colonisation

All enterovirulent *E. coli*

- 10.22. Many *E. coli* secrete a bacteriocidal colicin which has cytotoxic, receptor binding and translocation domains. A structural and functional analysis of protein interactions of colicin A and colicin E9 with their translocation proteins was carried out (**BBSRC BBD0163201**).

Host responses

- 10.23. The host response to infection with enterovirulent *E. coli* includes activation of both immune and inflammatory mechanisms, with an influx of the appropriate cell type to the site of colonisation. Enterovirulent *E. coli* have developed a number of mechanisms to avoid normal host defence mechanisms.

VTEC

- 10.24. VTEC, together with a range of other pathogens show tropism for the surface of the organised lymphoid tissue of the gut and the pathogen is then able to modulate host responses and compromise mucosal anti-bacterial immunity. This tropism was investigated by assessing the effects of EHEC on dendritic cell function in human Payers patches and with the myeloid dendritic cells (**BBSRC BBSB08566** followed by **BBSB085662**).
- 10.25. The mechanisms by which neurochemicals tyramine and norepinephrine (NE) produced by the host under stress or consumed in the diet contribute to the virulence of EHEC and *Salmonella* was investigated (**BBSRC BBSEI00001195** and **BBSRC BBC5180221**).
- 10.26. The influence of lymphostatin, which acts both as an inhibitor of lymphocyte function and as an adhesion molecule *in vitro*, on intestinal colonisation was analysed using EHEC-interactions with bovine epithelial and lymphoid cells (**BBSRC BBSEI00000952**).
- 10.27. A better understanding of the molecular basis of virulence of EHEC serovar O157:H7 in intestinal colonisation of ruminants was the aim of project **BBSRC BBSEI00001209**. The colonisation ability of strains of EHEC O157:H7 with deletions of specific virulence genes was tested. Strains were given orally to calves and the course of faecal excretion of the bacteria was monitored for 14 days.

EPEC

- 10.28. An animal model of colitis used the protein intimin of enteropathogenic *E. coli* (EPEC) transfected into the mouse pathogen *Citrobacter rodentium*, and a number of different knockout mice, were used to investigate the pathways by which activated T cells remodel colonic mucosa and to

determine which were the critical molecules *in vivo* (**BBSRC S11925**).

Identification of new factors involved in the pathogenicity of *E. coli*

10.29. The advent of genetic mining techniques, molecular biology, and proteomics together with the availability of the genome sequence map for *E. coli* has provided the tools required for the identification of new virulence factors.

VTEC

10.30. The factors mediating colonisation of cattle by enterohaemorrhagic *E. coli* were identified using the interactions of EHEC and specific EHEC mutants with bovine intestine in *in vitro* organ culture and with bovine intestinal mucosa *in vivo* (**BBSRC BBSEI00000715**).

10.31. An attempt to identify new additional colonisation and survival factors for EHEC was undertaken (**BBSRC D14378**). Signature-Tagged Mutagenesis of the mouse pathogen *C. rodentium* was used, and the function of the product of any identified genes was assessed using *in vitro* organ culture of intestinal mucosa.

10.32. Signature tagged and directed mutagenesis was also used to identify the contribution of non-LEE encoded factors of EHEC O157:H7 in colonisation and persistence (**DEFRA OZ0713**). The relationship between concurrent infection with parasites and enhancement of the colonisation and persistence of *E. coli* O157:H7 in sheep was also investigated.

All enterovirulent *E. coli*

10.33. A nuclear magnetic resonance (NMR) investigation of infection was performed in project **BBSRC JE514316** where virulence factors were structurally characterised and mechanisms of function proposed for key bacterial pathogens including *E. coli*, *Salmonella* Typhi, *Helicobacter pylori*.

Genomic studies of *E. coli*

VTEC

10.34. Analysis of the *E. coli* O157:H7 complete genome sequence has indicated that *E. coli* O157:H7 contains 1387 genes that are not present in the *E. coli* K-12 laboratory strain. Approximately 60% of these *E. coli* O157:H7-specific genes present in pathogenicity islands have no known function<sup>18</sup>.

10.35. A microarray of the complete *E. coli* O157:H7 genome was used to study the prevalence of O157-specific genes in other EHEC serovars and to

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<sup>18</sup> <http://www.nature.com/nature/journal/v409/n6819/full/409529a0.html>

analyse the transcription of EHEC genes *in vitro* and *in vivo* (**BBSRC BBSEI000001013**).

All enterovirulent *E. coli*

- 10.36. A functional genomics study of motility and associated regulons significant in environmental survival and persistence was carried out using the known genome sequences of multiple *E. coli* pathotypes (**BBSRC BBE01044X1**). Pathotypes were compared and the diversity of function of transcription networks within 'wild' members of the species was investigated to obtain a clear view of the relevance of the K-12 lab model as a paradigm for the species.
- 10.37. The sequence of the genome of enterotoxigenic (ETEC) *E. coli* H10407 was the final pathotype of *E. coli* to be completed (**BBSRC BBC5100751** and **BB/C510508/1**). Phylogenetic distribution of ETEC was also determined using multilocus sequence typing and strains of ETEC representative of each phylogenetic cluster were then examined by whole genome PCR and microarray to determine gene content and genetic structure.
- 10.38. A multi-level systems biology approach was used (**BBSRC BB/F003463/1**) to describe how a number of functional network modules emerge from a mathematical model of *E. coli* respiratory adaptation to oxygen. The model was then enriched by incorporation of new "omic" data.
- 10.39. Whole genome sequence information of *Salmonella*, *E. coli*, *Campylobacter* and *Streptomyces* was mined in order to identify key questions such as; which genes do bacteria switch on to cause food poisoning or in response to environmental signals, and which bacterial virulence genes are induced by novel environmental factors relevant to disease (**BBSRC BBSEF00041407**).
- 10.40. A computational methodology was used to generate inferences on the causal relationship between genes, proteins and metabolites, and to reconstruct transcriptional and metabolic networks representative of the response of *E. coli* to acid stress (**BBSRC BB/C515104/1**).
- 10.41. The development of xBASE, a series of taxon-specific databases with additional analytical and visualisation tools is a follow on project to the *E. coli* BASE website and database dedicated to comparative genomics of *E. coli* and related bacteria of relevance in the AgriFood context (**BBSRC BBE0111791**).

#### Regulation of gene expression

All enterovirulent *E. coli*

- 10.42. *E. coli* contain CreBC which is a regulator of the expression of genes whose products are involved in intermediary metabolism. The extent to which

CreBC influenced gene expression was examined using mutants of known CreBC activity together with the roles of two metabolic (environmental) signals that mediated the control of cre regulon gene expression by CreBC (**BBSRC BB/C514266/1**).

## 11. Assays for detection, typing and diagnosis of enterovirulent *E. coli*

### VTEC

- 11.1. Atypical sorbitol-fermenting (SF) strains of *E. coli* O157:H7 have recently emerged in the UK and cannot be identified on the standard detection media. Investigations have found that SF *E. coli* O157:H7 possesses the ability to bind and accumulate a dye, and when plated out forms red colonies. This capability was used to optimise a medium-based detection procedure alongside a multiplex polymerase chain reaction method for the detection of virulence genes (**FSA B11013**).

All enterovirulent *E. coli*

- 11.2. Next generation microarray technology was used to develop new, versatile and broadly applicable arrays for both expression and ChIP-on-chip (transcription factor binding to the genome) studies for multiple *E. coli* genomes (**BBSRC BBF00396X1**). Intact mRNA was isolated and amplified from *E. coli*, *Salmonella* Enteritidis and *Listeria monocytogenes*. However, further investigations found that the presence of mRNA did not correlate well with a measure of cellular viability (**Defra URG/001/99**). Current Methods and Monitoring Strategies on *E. coli* and Total Coliform Measurements (collaboration with American Water Works Association Research Foundation (AWWARF)) were also investigated (**Defra WT02059**).

### EAEC

- 11.3. The Enteroaggregative *E. coli* (EAEC) are an emerging cause of persistent and acute diarrhoea in humans. Infections caused by EAEC are under-diagnosed due to difficulties in differentiating the organisms from other *E. coli* and the considerable heterogeneity within the category. For the same reasons, very little is known about infection vehicles or reservoirs for EAEC. Development of a range of diagnostic tests for the detection of enteroaggregative *E. coli* (EAEC) included methods using genetic identification of conserved markers specific to EAEC, detection of known specific genes using multiplex PCR method, and also tests to identify the protein dispersin (**FSA B14002** and **B14003**).



## 12. Surveillance and management of VTEC in the food chain

- 12.1. This section is subdivided and begins by describing projects concerned with agricultural land and water. Subsequent sections span meat production and processing from farm to food production, and conclude by describing projects concerning consumer practices. Projects which deal solely with surveillance data are not included in this report.

### Agricultural land and water

- 12.2. The survival of *E. coli* O157 in soil at New Deer, Aberdeenshire was investigated by researchers involved in project **FSA Scotland S01004**.
- 12.3. Modelling of *E. coli* transport through soil/agricultural systems estimated the risk of faecal pathogens reaching surface or groundwater bodies. A geographical information system (GIS) based screen was then used to investigate the risk of diffuse (true non-point source) pollution to water bodies within different national catchments (**SEERAD QSR/002/03**).
- 12.4. The restocking of farm land (following a Foot and Mouth outbreak) was used to monitor the impact of changes in stocking density on the levels of total coliforms, faecal coliforms and enterococci from a range of water sources. Tentative relationships between stock density and faecal indicator organism concentrations were developed (**Environment Agency SCO20045SR**).
- 12.5. Information on the use of water in UK agriculture (irrigation, produce washing, drinking supplies, pesticide and fertiliser applications and glasshouse production) and the associated data on pathogen levels in agricultural water was collated and used to assess the risks to food safety from the presence of pathogenic micro organisms in farm water supplies (**FSA B17001**).
- 12.6. Strategies for reducing the risk of *E. coli* O157 infection to people in rural communities are being developed following the identification of some key factors involved in the spread of human food-borne pathogens in these environments. This will lead to the production of detailed risk management strategies on rural *E. coli* O157 infection to inform government policy (**Research Councils UK RELU-01**).

### Milk production

- 12.7. Transmission of VTEC can potentially occur from a contaminated farm environment to food stuffs. The prevalence of pathogens, including VTEC O157, in unpasteurised milk was determined by sampling in-line milk filters from 100 commercial dairy herds. The consumption of raw milk by farm families was also surveyed to obtain evidence of exposure to potentially harmful micro organisms (**FSPB 00-RESR-046**).

- 12.8. In order to generate a correlation between bacterial counts in milk and dairy hygiene inspectorate farm audit scores pathogen markers in raw milk have been assessed for their effectiveness as indicators of farm hygiene (**FSA B12002**).

#### Primary meat production

- 12.9. Primary meat production involves the rearing of the farm animals, the transport of livestock and the initial production of meat carcasses at the abattoirs. Of the cattle positive for *E. coli* O157:H7, a minority excrete large numbers of the bacteria in their faeces and these animals are considered to be essential for the transmission and maintenance of infection. It is thought that successful prevention of the maintenance of *E. coli* O157:H7 in the cattle population can be achieved by treating colonised and high-shedding animals. The major area where *E. coli* O157:H7 colonises cattle is the surface of the rectum and the removal of bacteria from this region can result in a massive reduction in numbers passed in the faeces<sup>19</sup>.
- 12.10. The immunology of endemic infectious diseases in ruminants was investigated with a focus on the transmission of VTEC. Research included, the role of the host, and of the verocytotoxin encoding bacteriophages and the application of immunological and genomics tools to investigating VTEC infections (**DEFRA VT0102**).
- 12.11. The prevalence and frequency of high level *E. coli* O157 and non-O157 VTEC in the faeces of sheep presented for slaughter in Scotland was investigated by microbiological sampling (**FSA Scotland S14005**). The data was used in the refinement of risk assessments for infection in humans and for comparison with prevalence estimations for cattle for both *E. coli* O157 and non-O157 VTEC.
- 12.12. Finished beef cattle on commercial farms were screened for *E. coli* O157:H7 (**DEFRA OZ0714**) and other non-O157 VTEC serogroups (O26, O103, O111, AND O145) (**FSA Scotland S01014**). The impact of antiseptic treatment of the rectum to reduce the prevalence rate and shedding levels of *E. coli* O157:H7 was measured (**DEFRA OZ0714**) and seasonal variation patterns were explored (**FSA S01014**). A further investigation examined the two routes of transmission of the bacteria; direct contact (via external body surfaces) or via environmental surfaces contaminated with faeces containing *E. coli* O157:H7.
- 12.13. In 2002 legislation in Northern Ireland required red meat carcasses to be microbiologically tested using a destructive excision protocol. Alternative carcass sampling protocols for cattle, sheep and pigs were evaluated to generate equivalent non-destructive methods for adoption throughout the UK (**DARD 0145**).

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<sup>19</sup> [http://randd.defra.gov.uk/Document.aspx?Document=OZ0712\\_7089\\_FRP.doc](http://randd.defra.gov.uk/Document.aspx?Document=OZ0712_7089_FRP.doc)

- 12.14. Key areas which increased the risk of the spread of contamination with food-borne pathogens were identified at various points along the lamb production/processing chain (on-farm management practices as well as transport, marketing and lairage factors). This led to the development and evaluation of intervention methods to control the spread (**FSA M01015**).
- 12.15. An examination of the effects of dietary and transport factors and of straw bedding and clipping on the cleanliness of cattle presented at the abattoir identified the most significant factors affecting faecal shedding of potentially zoonotic bacteria (**FSA M01013**). The findings of this and an earlier project (**FSA M01009**) were disseminated to the wider industry.
- 12.16. Using information collected on the epidemiology of VTEC O157 in cattle through project **DEFRA OZ0138**, the effect of various control measures was examined and a model developed which simulated transmission of VTEC within a beef farm (**DEFRA OZ0145**).
- 12.17. Project **VT0103 (DEFRA)** investigated the field of zoonotic pathogen evolution, transmission and control, the epidemiology of human *Campylobacter*, and the role of wildlife as reservoirs of zoonotic pathogens (*Campylobacter* and VTEC). Methods included modelling and network-theory to describe the complex interactions that drive the dissemination of pathogens through populations. Veterinary Research Training fellowships (**DEFRA VT0101**) also provided training to veterinarians to improve their awareness of the concept and practices of epidemiology.
- 12.18. Various electronic systems in Europe and the rest of the world record the results of anti- and post-mortem meat inspections in beef, sheep and pig abattoirs. A review assessed these data capture systems against criteria developed to meet UK data collection requirements (**FSA M01031**).

#### Strategies to manage *E. coli* levels in farm animals

- 12.19. As farm animals are the main reservoir for VTEC an additional way of reducing the risk of contaminated meat and thus risk to human health, may be to reduce the levels of bacteria which reside within the animal. A number of strategies have been described.
- 12.20. Vaccines based on two factors that influence intestinal colonisation by EHEC, intimin and lymphostatin were assessed for their possible potential to control EHEC colonisation in cattle in two projects (**BBSRC 1028** and the related project **BBSEI00001028**). A vaccine and its delivery system was also developed and investigated based on the antigens Loc8 fimbrial adhesin, the H7 flagellin and EspA type III translocation filaments, all expressed by EHEC O157:H7 (**DEFRA LK0666**).
- 12.21. The effect of engulfment by protozoa on the subsequent behaviour of verotoxigenic *E. coli* was investigated. In particular, whether engulfment

afforded greater protection after subsequent release of the bacterium through induction of a cross-protection phenomenon (**DARD 0260**).

- 12.22. The molecular mechanisms by which a selection of salicylanilide compounds reduced pathogenicity by disabling specific virulence factors was investigated as a method of limiting the effects of the *E. coli* Type III secretion system (TTSS) (**BBSRC BBD0106321**).

All enterovirulent *E. coli*

- 12.23. Plant-derived antimicrobially active components were extracted from hydroponically produced plants. Subsequent animal trials were conducted on up to 3 compounds which were tested in for their ability to reduce the load of pathogens *E. coli* and *Listeria* (**BBSRC BBSEG00006275**).

### Food Processing

- 12.24. Meat processing follows on from primary production and involves the application of Hazard Analysis and Critical Control Points (HACCP) principles. This is a systematic preventive approach to food safety that addresses physical, chemical, and biological hazards as a means of prevention rather than reliance on finished product inspection or testing. HACCP is used in the food industry to identify potential food safety hazards, so that key intervention points known as Critical Control Points (CCP's) can be used to reduce or eliminate the risk of hazards being realized. The system is used at all stages of food production and preparation processes including packaging, distribution, etc. Further discussion of HACCP and other aspects of risk can be found in the MSFFG report on Risk Assessment which covers other bacterial species in addition to *E. coli*<sup>20</sup>.
- 12.25. The microbiological verification of HACCP in meat plants was assessed with a review of the literature on methods for measuring microbiological load on carcasses and cut meat, and their suitability for verifying that measures taken at CCPs reduce the risk of contamination by enteric pathogens to acceptable levels (**FSA M01014**). Data was also collated about best HACCP practices across Europe and an analysis drew comparisons between countries (**FSA M01030**).
- 12.26. The survival of VTEC O157 through the manufacture of cheese produced from unpasteurised milk was investigated by measuring the presence and number of VTEC O157 cells during the cheese making process following their inoculation into the raw milk starting material (**FSA B12008**).
- 12.27. Animal by-products are classified into three groups (Category 1-3) according to the risk they present to public health. Category 3 materials can be used to produce animal/pet food but there is no requirement for marking

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<sup>20</sup><http://www.food.gov.uk/science/research/researchinfo/foodborneillness/microfundere/msffg/msffgriskassessmentreport>

this material, so it could be fraudulently used in the production of human food. A set of criteria were developed for potential markers which could be used for the detection of Category 3 animal by-products in human food products (**FSA M01026**).

#### Food outlets

- 12.28. Butchering practices used to prepare steaks may introduce micro-organisms onto the surface of the meat, even though the inner tissue area is considered sterile. Research investigated the microbiologically safety of rare steaks with an uncooked centre with respect to bacterial pathogens including *E. coli* (**Meat and Livestock Commission MLC 01**)<sup>21</sup>.
- 12.29. A comparison of businesses with a history of food poisoning outbreaks to those which had not experienced any outbreaks examined the factors beyond food safety control failures; i.e. the underlying management, operational and commercial systems, to help further explain why outbreaks occur (**FSA E03004**).

#### Practices in the home

- 12.30. The risks of food-poisoning from consumer practices can be divided into two areas; the consumption of products with a high potential bacterial burden and practices in the home that can contribute to food poisoning. A MSFFG report "*Food preparation practices and behaviour in relation to the microbiological safety of food*" has been published which covers other bacterial species in addition to *E. coli*<sup>22</sup>.
- 12.31. The levels of pathogens including *E. coli* O157 and *Campylobacter* spp were measured on kitchen cloths (a potential vehicle for cross-contamination) taken from households (**FSA B02015**).

### **13. Epidemiology of human VTEC and other enterovirulent *E. coli***

- 13.1. Epidemiology is the study of the distribution and causes of diseases within a population. Epidemiological studies can be used for a number of descriptive purposes; surveillance of the occurrence of the disease, study of disease progression, identification of prognostic factors and evaluation of treatments (Raymond *et al*, (2005)).

All enterovirulent *E. coli*

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<sup>21</sup> <http://www.msffg.org.uk/project.php?pid=1064>

<sup>22</sup> <http://www.food.gov.uk/science/research/researchinfo/foodborneillness/microfundamentals/msffg/msffgan09>

- 13.2. A report of the Department of Health funded IID (Infectious Intestinal Disease) Study in England by the Food Standards Agency (FSA) in 2000 defined disease burden, and calibrated national surveillance systems, (an estimation of the factor by which the number of cases of infection with specified pathogens needed to be multiplied to establish the actual number of infections in the community). Since this original study was undertaken, several structural changes have occurred in national surveillance. To repopulate the calibration with contemporary data a second IID study (**FSA B18021**) was commissioned to estimate prospectively the burden and causes of IID in the population and to compare the results with national surveillance data.
- 13.3. The relationship between virulence genotype, strain genotype and phenotype was investigated and defined for a number of non-O157 VTEC strains which had been isolated from animals, foods and human infections (**HPA 2005219**).

#### 14. Antibiotic resistance

- 14.1. A search of the database revealed a number of projects in which antibiotic resistance in *E. coli* was investigated. With the exception of one project, all projects that were identified in the *E. coli* search have been discussed within the March 2007 report from the Microbiological Safety of Food Funders Group on *UK publicly funded research on microbial antibiotic resistance in relation to the safety of food*<sup>23</sup>. These projects are listed in Appendix 3 but will not be further described here to minimise duplication of information.
- 14.2. One mechanism of antibiotic resistance is caused in part by bacterial efflux pumps which are membrane proteins that can pump a wide variety of antibiotics out of bacterial cells, and quickly adapt to recognise new antibiotics. The folding and assembly of small multi-drug transport proteins (SMRs) focusing on the proteins from *E. coli* (EmrE), *Mycobacterium tuberculosis* (TBsmr) and *Pseudomonas aeruginosa* (PAsmr) was investigated by examination of their response to a diverse array of drugs (**BBSRC B19845**).

#### 15. Risk assessment

- 15.1. The search of the database revealed a number of projects (see Appendix 4) which looked at *E. coli* O157 in relation to risk. These have been captured in a report from the Microbiological Safety of Food Funders Group on *UK publicly funded research relating to risk assessment and the microbiological safety of food*<sup>24</sup>.

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<sup>23</sup> <http://www.msffg.org.uk/reports/antibioticresistance.html>

<sup>24</sup> <http://www.food.gov.uk/science/research/researchinfo/foodborneillness/microfundere/msffg/msffgiskassessmentreport>

## 16. Avian pathogenic strains

- 16.1. Avian pathogenic *E. coli* (APEC) are key to the cause of colibacillosis, a severe systemic disease in poultry which can significantly impact on the poultry industry. The avian pathogenic *E. coli* factors that mediate carriage and virulence of APEC in poultry are poorly characterised, few genetic traits have been identified that delineate virulent and avirulent strains, and the role of such genes in pathogenesis in food-producing animals has rarely been tested. A number of studies (for example Moulin-Schouleur *et al* (2007), Zhao *et al* (2009)) have been published which describe the similarities and differences of APEC to other human extra-intestinal pathogenic *E. coli*, but currently there has been no link established between APEC and food consumption.
- 16.2. APEC genes, mediating respiratory tract colonisation and systemic virulence in turkeys, were identified and surveyed for the prevalence of APEC virulence factors. The virulence factors, in particular those predicted to be surface-exposed, were investigated by cloning, expression and purification. Their ability to induce cross-protective immunity against colibacillosis was also examined (BBSRC BB/E001661/1 and BBS/E/I/00001291).

## 17. Gaps in current MSFFG funded research

- 17.1. As described in Section 9, this report identifies groupings of a) basic fundamental projects on enterovirulent *E. coli* and b) applied projects with a focus on VTEC in relation to food safety.

### Basic Research

- 17.2. A key theme in the basic research projects is the use of molecular biology technologies. As predicted in the 2004 VTEC report, the availability of the complete genome sequence of *E. coli* K12,<sup>25</sup> and, more recently, the enteropathogenic strains O42 and O127:H6 strain E2348/69<sup>26</sup> has had a significant impact on both the technical approaches and data which projects now have available to use in their investigations. Projects have used genomic methods extensively, often in combination with structural and/or functional analysis, for example to investigate the effect of regulating factors on the expression of virulence genes at the molecular level. Gene sequencing and whole genome mining has also been undertaken, as has a comparative genomics study of a number of pathotypes and the K12 lab model. A number of projects also describe genomic searches for novel virulence factors. Interestingly, substantially less work has been undertaken on induction of virulence genes into commensal *E. coli*.

<sup>25</sup> <http://www.genome.wisc.edu/>

<sup>26</sup> [http://www.sanger.ac.uk/Projects/Escherichia\\_Shigella/](http://www.sanger.ac.uk/Projects/Escherichia_Shigella/)

- 17.3. The work described in this and previous VTEC report(s) has improved our understanding of *E. coli* pathogenicity. However, a number of gaps still exist:
- The attaching and colonisation mechanism in colonised, asymptomatic calves and how this compares to the findings in humans.
  - The reasons VTEC adhere to some species and not others.
  - Resistance to VTEC in humans and the role of asymptomatic carriers, particularly in relation to age and dose.
- 17.4. This list highlights what could be considered as gaps bridging the divide between basic understanding and the animal reservoir – host system. Such projects could be described as translational, using the knowledge obtained from basic research with genes and cells and linking it to pathogenicity in the animal reservoir and in humans. Additional information describing the benefits of translational research is available in the publication *Systems biology: a vision for engineering and medicine*<sup>27</sup> Within the field of *E. coli* research studies could investigate, for example, differences in growth of VTEC in the laboratory and *in vivo* or the expression of new virulence factors in the animal host. The gap in research relating to the human immunological response to VTEC and other *E. coli* strains could also be captured by translational studies. The knowledge obtained could be of great value if it was translated into therapeutic preventions or could identify biological differences between people who become infected and those which can carry VTEC.

#### Detection and Identification

- 17.5. The 2004 VTEC report identified the following areas in the context of this report:
- The need for an agreed, validated and robust set of primary isolation and identification protocols for use in research projects involving the detection of a wide variety of serovars of *E. coli*. This is especially important for VTEC and enteroaggregative *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 methods used for *E. coli* O157.
- 17.6. Current projects describe the development and use of Polymerase Chain Reaction (PCR) for identification of virulence determinants. PCR as a method, in combination with the use of microarrays, may therefore at some point supersede the use of serotyping. As PCR methods are still being developed, along with the identification of new virulence factors, there remains a need to develop both standardised protocols for the detection of enterovirulent strains and a system of identification. This is particularly

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<sup>27</sup> A pdf copy of this publication is available at <http://www.acmedsci.ac.uk/p99puid97.html>



important for non-O157 strains which can be less easy to detect with current methods. The Public Inquiry report into the South Wales 2005 outbreak of *E. coli* O157 recommended Variable Number Tandem Repeat (VNTR) should be validated as a standard method for the typing of *E. coli* O157<sup>28</sup>.

### Applied Research

- 17.7. Projects summarised in this report focused on identification of the sources of VTEC infection, for example agricultural water, farm animals and their contamination of the environment, and then look to improve, or put in place risk management strategies. Mathematical models and geographical information systems have also been used, which suggests a multidisciplinary approach to assessing sources of infection and epidemiology. This report, together with the previous 2004 VTEC report, also shows that the transfer of VTEC from the farm to the abattoir and strategies to reduce it has been investigated and some interventions identified. In addition, the Public Inquiry report into the South Wales Outbreak<sup>27</sup> highlighted the need to look into the feasibility of identifying and managing “supershedder” cattle on farms as a potential means of reducing the likelihood of spreading *E. coli* O157 to other cattle. Also of importance is the need to continue to investigate means of eliminating or controlling *E. coli* O157 in livestock, for example with vaccines or using bacteriophage or competitive exclusion.
- 17.8. There remains a lack of data on how VTEC survives during different stages of the food supply chain, including the preparation, preservation and treatment of food.
- 17.9. In the 2004 VTEC report it was suggested that with 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 parts of the food supply industry, including farmers. Reports described here have successfully included surveys and data gathering from user groups in drawing their conclusions. Further information is contained in the MSFFG report on *UK publicly funded research relating to food preparation practices and behaviour*<sup>29</sup>.
- 17.10. Much of the focus of VTEC monitoring and assessment has been on animals, with fewer projects focussing on human epidemiology. There remain gaps in areas such as the causes of variation in infections in man and the long-term consequences of infection following acute phase infection complications such as TTP and HUS. Such research could go some way towards addressing the human epidemiology in the context of VTEC pathogenicity.

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<sup>28</sup> <http://wales.gov.uk/ecolidocs/3008707/reporten.pdf?lang=en>

<sup>29</sup> <http://www.msffg.org.uk/reports/reports/foodprep.pdf>

## 18. Conclusions

- 18.1. The projects described in this report provide data and information on the fundamental biology of the pathogenicity of enterovirulent *E. coli*, and the identification and reduction of VTEC in the food chain in the context of human health. Projects tend to focus on either basic or applied research and there are similar numbers of projects distributed between these two areas. Initially the report aimed to review two sets of projects; those describing research into VTEC and those relating to non-verocytotoxin producing enterovirulent *E. coli*. However, on closer examination of project information it is apparent that although many projects do not specifically describe VTEC as the organism of interest, the data and findings of these studies are relevant to VTEC in addition to other enterovirulent *E. coli*.
- 18.2. Greater insights into the function and regulation of virulence genes have been developed especially in relation to how they are affected by the environment, preventative agents, and regulator genes. Additionally, identification of additional putative virulence factors and comparative genome studies has been possible due to the availability of genome sequences. The classification of enterovirulent *E. coli* has been influenced by the increased use of molecular methods, e.g. PCR which can identify virulence factors/genes and relate the information back to the different strains. The use of this technology, together with the potential discovery of new virulence factors does suggest that the classification system is still evolving. However, there will be a need to standardise classifications within the foreseeable future.
- 18.3. In general the colonisation of farm animals with VTEC and the contamination of farming environment are well established in terms of research. Those projects which are investigating VTEC with respect to food production and public health have a broad research base, assessing VTEC from farm to food production to consumer behaviour, together with other project concerned with, for example, reduction strategies, vaccines, and human epidemiology.
- 18.4. This report demonstrates that current research on enterovirulent *E. coli* has a strong basic research base, and that there is a good understanding of VTEC in relation to food production. This could be strengthened by using the information gained from fundamental molecular studies to further our understanding of the differences in behaviour of VTEC in cattle and other animals compared to humans. This would involve developing suitable *in vivo* models and taking a strongly molecular approach to understanding the colonisation events in different species. In addition, the rapid increase in availability of molecular data would benefit from a scheme to pool resources in this area.

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## Appendix I: Glossary

### Adhesin

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

### 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. The genes involved in the A/E lesion formation are found in the LEE pathogenicity island.

### Bacteriophage

Virus that infects a bacterium.

### Colicin

A globular proteinaceous toxin produced by some *E. coli* which is lethal to other closely related strains.

### ChIP-on-chip

A technique that combines chromatin immunoprecipitation (ChIP) with microarray technology (chip) to identify binding sites of DNA-binding proteins.

### Colibacillosis

An infection with *E. coli*, which occurs as an acute fatal septicaemia or sub-acute pericarditis and is a common systemic disease of poultry.

### *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*.

### *Etra, Eivf*

Two regulator genes which exert an effect on gene transcription in the LEE.

### Fimbriae

A proteinaceous appendage up to several micrometers long, used by bacteria to adhere to one another and to adhere to animal cells.

### **Flavo-haemoglobin**

A metal binding globular protein found in bacteria, which is thought to play a role in nitric oxide metabolism and/or transport.

### **Genome, genomic, genotype.**

The **genome** encompasses both the genes and the non-coding sequences of the DNA of one set of chromosomes. The **genotype** is the genetic constitution of a cell, an organism, or an individual. **Genomics** is the study of the genomes of organisms and includes determining and analysing the DNA sequence of organisms and fine-scale genetic mapping.

### **Genetic mining**

The extraction of explicit, and potentially interesting, information from large amounts genome data, often using complex algorithms and neural networks.

### **GFP (Green fluorescent protein)**

A protein which fluoresces when exposed to blue light and can be synthesized by a cell following insertion of the GFP gene into its genome. GFP is often inserted alongside a gene of interest. When transcription occurs to both genes, GFP is produced and can be visualized and *reports* that the gene of interest has also been transcribed.

### **HACCP (Hazard Analysis and Critical Control Point analysis)**

A systematic preventive approach used in the food industry to identify potential food safety hazards, so that key actions, known as Critical Control Points (CCPs) can be put in place to reduce or eliminate the risk of the hazards occurring.

### **Haemorrhagic colitis**

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

### **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.

### **Knock-out mice**

A strain of mice that has been genetically engineered such that the function of a specific gene has been disrupted. Often used to study the function of the gene that has been "knocked-out" of the DNA.

### **KpsD**

A gene which encodes a periplasmic protein which is involved in the transport of bacteria coat proteins across the bacterial membrane.

**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.

**ler (LEE encoded regulator)**

The *ler* gene is part of the LEE operon. The *ler* protein is a regulator which attaches to the promoters of the other LEE genes and caused their transcription.

**Lymphostatin**

A protein produced by EPEC that inhibits host lymphocytes from proliferating and producing cytokines.

**mRNA (messenger Ribonucleic acid)**

Single stranded RNA which contains the genetic code from which proteins are synthesised at the site of the ribosome.

**Metallomics**

The study of metal containing proteins.

**Microarray**

A multiplex technology consisting of an arrayed series of thousands of microscopic spots called features. Features can be for example, a short section of a gene or other DNA element that are used as probes to which related cDNA or cRNA sample will anneal. Hybridization is usually detected and quantified by fluorescence-based detection of fluorophore-labeled targets.

**Nitrosative stress**

Adverse effects occurring when the generation of reactive nitrogen species in a system exceeds the system's ability to neutralize and eliminate them.

**NMR (Nuclear Magnetic Resonance)**

A method which uses the resonance properties of atomic nuclei to study molecular physics, crystals and non-crystalline materials.

**Pet (Plasmid-encoded toxin)**

An autotransporter plasmid-encoded toxin (Pet) of enteroaggregative *Escherichia coli* (EAEC) which produces cytotoxic and enterotoxic effects on the host system.

**Proteomics**

The large-scale study of proteins present within a cell, or tissue, or an organism, particularly their structures and functions.

**Phenotype**

An observable characteristic of an organism.

**Phylogenetics, Phylogenetic cluster**

The study of the evolutionary relatedness among various groups of organisms (e.g. species, populations), which is discovered through molecular sequencing data and morphological data matrices.

**PCR (Polymerase Chain Reaction)**

A widely-used molecular technique to generate multiple copies of a target DNA sequence by amplification.

**RT-PCR (Reverse Transcriptase Polymerase Chain Reaction)**

A laboratory method commonly used in molecular biology which involves the amplification of an mRNA target sequence into a complementary DNA strand.

**Rcs**

The Rcs signal transduction system of *E. coli* regulates capsular polysaccharide synthesis (*cps*) genes.

**STM (Signature Tagged Mutagenesis)**

A technique where genes are altered with random insertions of small mobile DNA sequences (which are themselves tagged with a sequence for identification) into a genome. When the genes are transcribed and translated the protein structures are altered and loses function but can be traced via the tag. This method can be used as a means of identifying virulence genes in bacteria which are essential for the process of infection in a chosen animal model.

**Taxon specific database**

A database which contains metabolic pathway data for a small number of closely related species that fall within a relatively small taxonomic unit, such as a family.

**TTP (Thrombotic Thrombocytopenic Purpura)**

Formation of microscopic thromboses in small blood vessels throughout the body. Red blood cells passing through the microscopic clots are subjected to shear stress which damages their membranes, leading to intravascular haemolysis. Reduced blood flow due to thrombosis and cellular injury may result in organ damage.

**Tropism**

Specific movement in response to an environmental stimulus.

**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 demonstrate.

**VTEC O157**

Verocytotoxin-producing *Escherichia coli* of serogroup O157.

**Virulence cassette**

A deoxyribonucleic acid cluster commonly containing genes associated with pathogenesis. Also known as pathogenicity islands.



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## Appendix 2: Projects identified in the MSFFG database described in this report

Project code	Project Title	Funder	Start Date	End date
1028	Development of novel vaccination strategies for the control of enterohaemorrhagic <i>Escherichia coli</i> in cattle	BBSRC	01-Jun-03	31-May-04
B16926	Chaperone dependent assembly and structure of a bacterial polypeptide capsule	BBSRC	01-May-02	30-Apr-05
B17144	Visualisation of secreted translocator and effector proteins during EPEC and EHEC O157:H7 type III secretion	BBSRC	01-Sep-02	01-Sep-05
B19845	Assembly of multi-drug transport proteins; a fundamental aspect of antibiotic resistance of pathogenic bacteria	BBSRC	01-May-04	30-Apr-07
BB/C509807/1	Sialic acid transport in pathogenic bacteria: a novel role for TRAP transport systems	BBSRC	17-Jan-05	16-Jan-08
BB/C510075/1	Genomics of enterotoxigenic <i>Escherichia coli</i> (ETEC)	BBSRC	01-Jan-05	30-Apr-07
BB/C510508/1	Genomics of enterotoxigenic <i>Escherichia coli</i> (ETEC)	BBSRC	13-May-05	12-May-07
BB/C514174/1	Bacterial redox homeostasis: Roles of the ABC-type substrate exporter, CydDC	BBSRC	01-Sep-05	31-Aug-08
BB/C514266/1	Transcriptional networking through the Cre regulons of <i>Escherichia coli</i> and <i>Salmonella</i> Typhimurium	BBSRC	01-Jun-05	31-May-08
BB/C515104/1	Metabolic and transcriptional pathway inference using state space models: an application to understanding acid response	BBSRC	01-Dec-05	30-Nov-08
BB/E001661/1	Identification of avian pathogenic <i>Escherichia coli</i> genes required for carriage and virulence in poultry	BBSRC	11-Apr-07	10-Apr-10
BB/F003463/1	An integrative multi-level systems biology approach to understanding enterobacterial responses to oxygen	BBSRC	27-Aug-07	26-Aug-10
BBC5098311	An integrated post-genomic characterisation of the <i>Escherichia coli</i> metalome with special reference to zinc	BBSRC	07-Mar-05	06-Mar-08
BBC5167011	Regulation of gene expression within the locus for enterocyte effacement	BBSRC	01-Feb-05	31-Jan-08
BBC5180221	Influence of the neuroendocrine stress hormones on the carriage and virulence of zoonotic bacterial pathogens in farm animals	BBSRC	01-Apr-05	30-Nov-09
BBD0101951	The scatterlings of virulence: towards a complete type-III secretion effector repertoire in <i>Escherichia coli</i>	BBSRC	01-Feb-06	31-Jan-09
BBD0106321	Inhibition of bacterial Type III secretion by salicylanilides	BBSRC	12-Oct-06	11-Oct-09
BBD0163201	Structural and functional analysis of multiprotein complexes required for colicin translocation	BBSRC	01-Oct-06	30-Sep-09
BBE01044X1	Environmentally relevant responses in different <i>Escherichia coli</i> pathotypes: a functional genomics study of motility and associated regulons	BBSRC	01-Nov-06	31-Oct-09

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Project code	Project Title	Funder	Start Date	End date
BBE0111791	xBASE: a bioinformatics resource for the AgriFood bacteriology community	BBSRC	01-Feb-07	31-Jan-12
BBE0208601	Ler, a versatile global regulator from <i>E. coli</i> O157 and related strains	BBSRC	01-Jul-07	20-Jun-10
BBF00396X1	Design, optimisation and validation of high density microarrays for multiple <i>Escherichia coli</i> genomes	BBSRC	01-Oct-07	30-Jun-08
BBS/E/I/00001291	Identification of avian pathogenic <i>Escherichia coli</i> genes required for carriage and virulence in poultry	BBSRC	11-Apr-07	10-Apr-10
BBSB05265	Molecular characterisation of the processes responsible for toxigenic conversion of <i>E. coli</i> by VT-bacteriophages	BBSRC	02-Aug-04	01-Aug-07
BBSB08566	Immunological consequences of the interaction of enterohaemorrhagic <i>E. coli</i> with the organised lymphoid tissues of the gut	BBSRC	01-Apr-04	31-Jul-05
BBSB085662	Immunological consequences of the interaction of enterohaemorrhagic <i>E. coli</i> with the organised lymphoid tissues of the gut	BBSRC	31-Jan-06	30-Mar-08
BBSB09627	Capsular polysaccharide export in <i>Escherichia coli</i> : a determination of the role of the KpsD protein in export across the outer-membrane	BBSRC	01-Apr-04	31-Mar-07
BBSEF00041208	Molecular microbiology of <i>Salmonella</i> Typhimurium and <i>E. coli</i>	BBSRC	01-Apr-00	31-Mar-05
BBSEF00041407	Bacterial functional genomics: Dissemination of cutting-edge technology (BACFUN)	BBSRC	31-Jan-02	09-Apr-06
BBSEG00006275	Plant-derived antimicrobial activities for use in animal feed: (ERDF KEF)	BBSRC	01-Apr-05	30-Jun-06
BBSEI00000715	Identification of factors mediating colonisation of cattle by enterohaemorrhagic <i>Escherichia coli</i>	BBSRC	01-Oct-00	11-Apr-07
BBSEI00000952	Role of lymphostatin in the colonisation of cattle by enterohaemorrhagic <i>Escherichia coli</i>	BBSRC	01-May-02	30-Apr-05
BBSEI00001013	Genetic analysis of enterohaemorrhagic <i>Escherichia coli</i> colonisation and pathogenesis in cattle.	BBSRC	01-Apr-03	31-Mar-08
BBSEI00001028	Development of novel vaccination strategies for the control of enterohaemorrhagic <i>Escherichia coli</i> in cattle	BBSRC	01-Jun-03	01-Jan-06
BBSEI00001195	Influence of neuroendocrine stress hormones on the carriage and virulence of zoonotic bacterial pathogens in farm animals	BBSRC	01-Apr-05	31-Mar-08
BBSEI00001209	Intestinal colonisation of extracellular bacterial pathogens	BBSRC	14-Oct-03	13-Oct-05
BBSEJ0000A189	Virulence factor trafficking pathways in bacteria	BBSRC	01-Oct-04	30-Sep-09
BBSEJ0000A221	Functional genomic characterisation of the bacterial Tat complex as a nanomachine for biopharmaceutical production and a target for novel anti-infectives (Tat machine)	BBSRC	01-Nov-04	31-Oct-08
D12994	Expression and release of toxin and converting phage in Shiga toxin-producing <i>Escherichia coli</i> (STEC)	BBSRC	01-Apr-00	01-Apr-03
D14378	Intestinal colonisation factors common to <i>E. coli</i> O157 and other A/E lesion-forming luminal microbial pathogens	BBSRC	01-Feb-01	01-Feb-04

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Project code	Project Title	Funder	Start Date	End date
JE514316	The structural basis of infection: a nuclear magnetic resonance (NMR) approach	BBSRC	01-Mar-01	01-Mar-04
P14130	Characterisation of the Gram-negative bacterial autotransporter secretion mechanism	BBSRC	23-Apr-01	08-Sep-04
P15901	Responses of <i>Escherichia coli</i> to nitrosative and oxidative stresses in vitro and in macrophage: a post-genomic approach	BBSRC	01-Jul-02	30-Jun-05
P16371	Role of the Rcs two-component pathway in bacterial growth on solid surfaces and biofilm formation	BBSRC	01-Jul-02	01-Jul-05
P16414	New insights into bacterial outer membrane biosynthesis	BBSRC	01-Jul-02	01-Jul-05
P18939	Flavo-haemoglobin: a bifunctional NO-Detoxifying enzyme for facultatively aerobic bacteria	BBSRC	14-Apr-03	13-Jul-06
S11925	The mechanisms of T cell-induced mucosal growth in the gut	BBSRC	01-Jan-00	01-Nov-03
145	The development of protocols for the microbiological assessment of red meat carcasses, in the context of HACCP	DARD	01-Jan-03	31-Dec-03
DARD 0260	An investigation of the effect of protozoan engulfment of verotoxigenic, necrotoxigenic <i>Escherichia coli</i> and <i>Campylobacter jejuni</i> on their subsequent resistance to food processing operations	DARD	01-Oct-04	30-Jun-06
LK0666	Vaccination strategies for control of enterohaemorrhagic <i>Escherichia coli</i> O157:H7 in cattle	Defra	01-Oct-05	30-Sep-08
OZ0145	VTEC O157 on farm control: Effective measures, perception and risk communication	Defra	01-Dec-05	31-May-07
OZ0713	The Role of native host gut flora and innate immune status upon the colonisation of <i>E. coli</i> O157 in ruminants	Defra	01-Oct-04	30-Sep-07
OZ0714	To develop a cost effective and practical method to reduce <i>E. coli</i> O157 infection in cattle prior to slaughter	Defra	01-Jan-08	01-Jan-11
URG/001/99	Solid phase rapid detection of viable micro-organisms using nucleic acid amplification and biosensing techniques	Defra	04-Aug-00	04-Aug-00
VT0102	Integration of functional genomics and immunology and their application to infectious disease in ruminants	Defra	01-Apr-04	30-Sep-09
VT0103	Foodborne zoonotic pathogens: Transmission, pathogen evolution and control - a programme of training and research	Defra	01-Apr-04	31-Mar-09
B02015	A national survey of potential cross-contamination resulting from kitchen cloths in domestic kitchen	FSA	01-Aug-00	31-Mar-02
B11010	Review of past and current research on verocytotoxin-producing <i>Escherichia coli</i>	FSA	01-Jul-04	31-Mar-04
B11013	Development and validation of methods for the detection and identification of sorbitol-fermenting verocytotoxin-producing <i>Escherichia coli</i> O157	FSA	01-Oct-07	01-Feb-10

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Project code	Project Title	Funder	Start Date	End date
B12002	Review of raw milk analyses methods and assessment of effectiveness as pathogen markers and indicators of farm hygiene	FSA	01-Jul-02	30-Jun-04
B12008	Investigate the survival of <i>Mycobacterium bovis</i> and <i>E. coli</i> O157 in UK-produced cheeses made from raw cows' milk	FSA	01-Sep-07	01-Sep-09
B14002	Methods for the detection of Enteroaggregative <i>Escherichia coli</i>	FSA	01-May-02	30-Apr-04
B14003	Development and validation of diagnostic tests for Enteroaggregative <i>Escherichia coli</i>	FSA	01-Jan-02	31-Dec-04
B17001	A review of the use of water in UK agriculture and the potential risks to food safety	FSA	01-May-01	28-Feb-02
B18021	The second study of infectious disease in the community - determining disease burden and calibrating national surveillance data in the United Kingdom	FSA	01-Apr-06	31-May-10
E03004	Management risk factors resulting in foodborne disease outbreaks in the catering industry - a case control study	FSA	01-Oct-01	01-Mar-05
M01013	Farm management practices to improve the visible and microbiological cleanliness of cattle hides at slaughter	FSA	01-Jan-00	30-Sep-03
M01014	Microbiological verification of HACCP in meat plants	FSA	01-Apr-00	01-Apr-01
M01015	Factors affecting the presence and spread of human bacterial pathogens in sheep	FSA	01-Jan-00	31-Dec-03
M01026	A feasibility study into the marking of Category 3 (low risk) animal by-products	FSA	01-Aug-04	01-Jul-05
M01030	Global HACCP implementation in meat producing countries	FSA	01-Dec-03	01-May-04
M01031	Effective communication of animal health information through the supply chain for animals entering the slaughtering process	FSA	01-Oct-03	28-Feb-04
S01004	Survival of <i>E. coli</i> O157 in soil at New Deer	FSA	28-Aug-00	28-Feb-01
S14005	Prevalence and concentration of <i>Escherichia coli</i> serotype O157 and other VTEC in sheep presented for slaughter in Scotland	FSA	01-Jul-05	30-Sep-06
S01014	Prevalence of faecal shedding on Scottish beef cattle of verocytotoxigenic <i>Escherichia coli</i> serotypes: O26, O103, O111 and O145 (S01014)	FSA Scotland	01 Jan-02	31-Dec-03-
00-RESR-046	Detection and molecular characterisation of selected pathogenic organisms isolated in unpasteurised milk using milk filters.	FSPB	01-Jan-01	01-Sep-04
00-RESR-102	Food safety knowledge, microbiology and refrigeration temperatures in domestic kitchens on the island of Ireland	FSPB	1 Jun-02	30-Jun 05-
2005219	A study of the molecular epidemiology of non-O157 VTEC from animals, foods and human infections - farm to fork	HPA	01-Dec-04	31-Oct-07
MLC 01	Examination of the microbiological safety of rare steak	MLC	No dates available	No dates available
G0401551	Enterohaemorrhagic <i>E. coli</i> infection: Identification of the host cell intimin receptor (HIR)	MRC	01-Oct-05	30-Sep-08

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Project code	Project Title	Funder	Start Date	End date
G0500583	Assembly of the actin pedestals central to adhesion of enteropathogenic <i>E. coli</i>	MRC	01-Nov-05	31-Oct-08
RELU-01	Reducing <i>E Coli</i> O157 risk in rural communities	RELU	01-Oct-07	30-Sep-10
QSR/002/03	Provision of a screening tool to identify and characterise diffuse pollution pressures and impacts: phase II	SEERAD	22-Sep-03	24-Oct-03
SC020045SR	The impact of destocking on the microbiological qualities of rivers in the Caldeu catchment	Environment Agency	01-Sep-00	31-Dec-04
WT02059	Significance of current methods and monitoring strategies on <i>E. coli</i> and total coliform measurements (collaboration with AWWARF)	Defra	05-May-06	04-May-08

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**Appendix 3: Projects identified in MSFFG database but covered in Antibiotic Resistance report *UK publicly funded research on microbial antibiotic resistance in relation to the safety of food*<sup>30</sup>**

Project Code	Project title	Funder	Start Date	End Date
OD2001	An <i>in vivo</i> poultry model to study the effect of growth promoters in resistance to human antibiotics in mixed bacterial populations	Defra	01-Oct-00	30-Sep-03
OD2002	Field investigation of acquisition and persistence of multiple resistant <i>E. coli</i> in cattle and sheep exposed to farm waste	Defra	01-Apr-00	31-Mar-04
OD2004	Loss of antibiotic resistance: analysis of phenotype and related gene expression	Defra	01-Apr-00	31-Mar-03
OD2005	A laboratory and field study to assess the potential for transfer of antibiotic resistance between bacterial strains in stored and spread organic wastes	Defra	01-Apr-00	31-Mar-03
OD2006	Investigation of persistence of antimicrobial resistant organisms in livestock production	Defra	01-Oct-00	30-Sep-05
OD2007	Genetic characterisation of resistance markers in sentinel <i>Escherichia coli</i> and <i>Enterococcus</i> in farm animals	Defra	01-Apr-01	30-Sep-04
OD2008	Transfer of antimicrobial resistance genes between bacteria in stored and spread farm wastes	Defra	01-Sep-00	31-Aug-03
OD2009	Wild rodents as reservoirs of antibiotic resistance for farm animals and man-current status, origins and mechanisms of persistence	Defra	01-Oct-00	30-Sep-03
OD2022	Characterisation of antibiotic resistance gene silencing in <i>Escherichia coli</i>	Defra	01-Jan-06	31-Dec-08
VM02100	Factors influencing the development of resistance to fluoroquinolone antibiotics by foodborne bacteria	Defra	01-Apr-00	30-Sep-03
VM02105	Identification and use of genomic markers of antibiotic resistance in campylobacters, salmonellae and enterococci.	Defra	01-Aug-00	31-Jul-03
VM02136	Development of rapid response gene profiling for identification of antimicrobial resistance genes in enterobacteria from food animals and humans	Defra	01-Mar-03	31-May-06
VM02201	Modulation of dosing regimes to prevent development of fluoroquinolone resistance in bacteria (mainly <i>Salmonella</i> and <i>E. coli</i> ) in chicken	Defra	01-Apr-04	31-Mar-06
VM02203	Interventions to reduce the carriage of antimicrobial resistance in food producing animals	Defra	01-Apr-06	30-Nov-07
SAC/254/00	Antimicrobial resistance modelling	SEERAD	01-Apr-00	31-Mar-04

<sup>30</sup> <http://www.food.gov.uk/science/research/researchinfo/foodborneillness/microfundors/msffg/msffgmicrobialantiresist>

**Appendix 4: Projects identified in the MSFFG database but contained in the report entitled *UK publicly funded research relating to risk assessment and the microbiological safety of food*<sup>31</sup>.**

Project Code	Project title	Funder	Start Date	End Date
OD2023	Potential risk to human and animal health from the emergence and spread of beta-lactamase resistance in animals in Great Britain	Defra	01-Feb-07	31-Jan-10
OD2025	Antimicrobial use and carriage of antimicrobial-resistant <i>Escherichia coli</i> and staphylococci in dogs and horses in the community: molecular mechanisms of resistance and risk to humans	Defra	01-May-07	30-Apr-10
OD2026	The clinical treatment of pet dogs and antibiotic resistance in commensal and potentially pathogenic bacteria	Defra	01-Jun-07	31-May-10
M01025	An evaluation of the effect of EU proposals to inspect licensed premises on the marketing of wild game: a qualitative risk assessment.	FSA	01-Dec-02	30-Apr-03
BBSEF00041214	Quantitative risk assessment for microbiological food safety	BBSRC	01-Apr-00	31-Mar-05

<sup>31</sup><http://www.food.gov.uk/science/research/researchinfo/foodborneillness/microfundere/msffg/msffgriskassessmentreport>