

UK PUBLICLY-FUNDED RESEARCH RELATING
TO VEROCYTOTOXIN-PRODUCING
ESCHERICHIA COLI (VTEC)

**Report of the
Microbiological Safety of Food Funders Group
(MSFFG)**

INTRODUCTION

1.1 The primary aim of this paper is to provide an overview of publicly funded research relating to Verocytotoxin-Producing *E. coli* (VTEC). For the purposes of this paper “VTEC” refers to VTEC O157, unless otherwise stated. The details relating to funded projects have been provided by Members of the Microbiological Safety of Food Funders Group (MSFFG) who have considered the available information in order to make an assessment of the findings **up to the end of June 1999** and identify any gaps in funded research. In making its assessment the MSFFG has considered the views and recommendations of expert Groups such as the Advisory Committee on the Microbiological Safety of Food (ACMSF), the Pennington Group, and the World Health Organisation (WHO). Questions/considerations for VTEC 2000 (<http://www.liglobal.com.galler/vtec2000.html>) posed at the 3rd International Symposium and Workshop on Verocytotoxin-Producing *E. coli* Infections (Baltimore, 1997) were also considered. The MSFFG has defined gaps in research as “perceived lack of information”. Such gaps in research will be considered as possible priorities for additional work, but will not necessarily be addressed by Government funding.

1.2 The aim of this work is to also identify overlaps in publicly funded research to ensure a complementary and coherent research programme. In some instances work has been funded with similar titles, but examination of the ongoing work has revealed complementary rather than overlapping objectives.

1.3 It is not the aim of this paper to devise a strategy of control for VTEC infection, but the information presented will be used to inform a strategy for control.

1.4 In some instances the detailed Current State of Publicly Funded Research projects have not been included. This is where a project has only recently started and no details are yet available, or have not been reported for reasons of confidentiality. The paper has considered publicly funded research **from 1990 to the end of June 1999**. Table A1.3 lists new research that has started since July 1999 and this will be updated on a regular basis.

1.5 The information provided in this paper has been provided by members of the MSFFG or by their contractors. The findings presented have not necessarily been peer reviewed, although all contractors are encouraged to publish the findings of their research in peer reviewed literature.

1.6 The information on funded research has been considered under the following headings:

- Detection, differentiation and diagnosis
- Microbial physiology and genetics
- Pathogenesis
- Epidemiology
- Surveillance
- Risk analysis

- Reduction and elimination
- Microbial antibiotic resistance
- Other

Although the projects assessed have been considered under these defined categories, a lot of the research has been funded with multiple purpose, i.e. covering more than one research area. The paper does not aim to cover research associated with VTEC in water or direct contact with VTEC, but research in these areas which has implications for the microbiological safety of food has been referred to.

1.7 This overview paper on publicly funded research on VTEC represents one of the outputs of the deliberations of the MSFFG and the format will be repeated for other foodborne pathogens. These overview papers address the Group's terms of reference "*To assist the co-ordination of publicly funded research and development on the microbiological safety of the food chain with a view to informing the R&D effort, identifying gaps and overlaps, and providing reports as appropriate*".

2. DETECTION, DIFFERENTIATION AND DIAGNOSIS

Introduction

2.1 The ACMSF (1) made a number of recommendations for Government funded research in the area of laboratory methods for the detection of VTEC in clinical samples and food. It was recommended that research be undertaken into:

- *in vitro* methods for demonstration and detection of pathogenicity determinants to aid laboratory diagnosis
- the development and evaluation of different solid media for O157 VTEC
- rapid methods to detect VTEC of all serogroups and Verocytotoxin in food and clinical material
- the development of methods for improved sub-typing of VTEC and particularly O157 VTEC
- the evaluation of conventional and rapid methods for the examination of foods and environmental samples for O157 VTEC.

2.2 The Pennington Group (2) recommended research to improve the current DNA-based methods for the identification of *E. coli* O157.

2.3 The WHO (3) recommended the development of detection methods for EHEC to diagnose human disease and to determine occurrence in animals/food and general ecology, and the development of quantitative detection methods. The WHO also recommended that virulence factors which define EHEC as a human pathogen be identified.

2.4 The detection, differentiation and diagnosis category holds the largest number of projects of any one-area (22). Within this classification lie projects more principally related to a variety of other areas such as epidemiology, physiology, pathogenesis and surveillance. However, the largest group of projects relates to the development of detection methods.

Current State of Publicly Funded Research

2.5 A wide ranging study of the prevalence of *E. coli* O157 in the Sheffield area has examined faecal samples from animals at slaughter, faecal samples from patients with non-bloody diarrhoea and a variety of foodstuffs (**DH, 182**). It was found that almost all the strains isolated from sheep and cattle were verocytotoxigenic, *eaeA* positive, contained the 92 kbp plasmid and were thus typical of strains causing infections in man. In contrast, strains from pigs were found to be an unlikely source of human infection. Ongoing work is comparing the isolates from cattle and beef products and sheep/lamb products using a variety of molecular typing techniques (**DH, 182B**).

2.6 A project in Northern Ireland has involved the development of methodology for the sampling of beef carcasses to determine their microbial quality and has shown

hide pulling to be the principal critical control point (**DANI, 9723**). Of surveys being carried out in Scotland, there is one on beef cattle to determine the proportion of animals on positive farms infected with *E. coli* O157 and efforts are being made to correlate this to farm management practices (**SERAD, SAC/168/97**). There is also a survey to examine isolates from selected farms to determine what proportion are VTEC (**SERAD, UAB/006/97**).

2.7 Of the three projects which have studied adhesins, one is investigating *eaeA* negative VTEC isolates to identify the nucleotide sequence of the gene/s contributing to adherence (**DH, 251**). So far, 250 mutants have been screened for loss of adherence to Hep-2 cells and a total of ten mutants with reduced adherence have been identified. These are now being subjected to genetic analysis. In the others, SDS-PAGE of the outer membrane protein has enabled characterisation of defined proteins under various physiological conditions and adhesins are being used to develop a detection method for all VTEC (**DH, 246 & 248**).

2.8 A study examining the antibody response of patients infected with *E. coli* O157 has shown that enterohaemolysin and flagellar antigens are poorly antigenic or not expressed during infection (**DH, 242**). However, 70% of patients with serum antibodies to LPS make salivary antibodies to the antigen. Some non-culturable VTECs have been shown to produce toxins and this is being investigated further.

2.9 Epidemiological investigations include a project aimed at investigating whether the isolates from three Scottish *E. coli* O157 outbreaks could be distinguished using molecular techniques (**SERAD, LEP/001/97 & UAB/004/97**). Two laboratories independently reached the conclusion that the isolates could be distinguished and there is not a sub-clone which prevails in Scotland. Molecular techniques are also been developed to determine interrelationships in carriage between EHEC and non-EHEC strains isolated from ruminants (**BBSRC/SERAD, BFP11335**). In Wales, a project to assess the population burden and role of zoonotic spread of VTEC included following a cohort of farmers for 18 months and examining the prevalence of excretion of VTEC and seroprevalence of antibodies (**DH, 254**). 1.7% of serum samples were found to contain antibodies to *E. coli* O157. VT genes were isolated from 6.3% of faecal samples but all of the isolates were non-O157 serotypes. No illness was seen amongst the farmers during the study.

2.10 Two projects address VTEC typing. The first aims to identify and develop a series of simple, inexpensive tests to characterise VTECs on the basis of such factors as their tolerance to acid and survival on surfaces (**MAFF, FS3109**). The purpose being to develop a 'scoring' system to differentiate tolerant and sensitive strains. The objective of the second project is to develop a sensitive reproducible typing scheme using techniques such as RFLP, PFGE and PCR based tests to discriminate between strains (**MAFF, FS1238**).

2.11 Work being carried out to develop new detection methods utilises a variety of approaches. One project uses a molecular and immunological approach based on adhesins. It aims to design and validate a PCR for the specific detection of the *eae* gene in VTEC plus evaluate immunomagnetic, chromatographic and membrane grid

techniques for application to direct to samples or enrichment broths (**MAFF, FS1231**).

2.12 An alternative approach is the use of monoclonal antibodies to specific surface proteins to develop a sandwich ELISA technique for VTEC. Antibodies have so far been successfully raised to fimbrial antigens of O26 and O157, and work is now underway to raise antibodies to intimin (**DH, 246**). Additional studies aiming to improve techniques for the rapid detection of *E. coli* O157 in foods, faeces, soil and water are underway, but details of methods and progress are not yet available (**SERAD, UAB/005/97 and SERAD, UAB/007/99**). A generic, non-culture method for the detection of viable microorganisms, including *E. coli* O157, based on isolation and detection of bacterial RNA is currently being developed (**SERAD, URG/001/96**). The RNA isolation and amplification techniques have been developed and the RNA detection system is currently being evaluated.

2.13 Detecting the presence of mRNA using RT-PCR has the potential to discriminate living cells from dead cells. This was explored in a project which developed a single tube RT-PCR for detecting mRNA in *E. coli* and *Salmonella enteritidis*. It was found that at 37°C mRNA disappeared from dead cells within about 2 hours and the sensitivity of the RT-PCR was found to be 100 *E. coli* cells/ml when using pure cultures (**DH, 195**).

2.14 The novel use of liposomes in solid media is being utilised to produce a system whereby colour development within the media signals the presence of both VT production and the biological activity of VT1 and VT2. Optimisation of the technique is currently underway and the project will hopefully provide a simple, low cost test for identifying VTEC (**DH, 244**). Colour development is also the theme of a second project which aims to detect low numbers of VTEC in foods using antibody directed labelling of the organisms with luminescence-labelled nanoparticles (**SERAD, UMA/001/95**). Ultimately the aim is to develop a sensitive and direct test for the enteropathogenic organisms without the time consuming requirement for culture.

2.15 The present status of *E. coli* O157, in terms of detection and diagnosis, is currently being evaluated as part of a funded project (**MAFF, OZ0141**).

MSFFG Assessment and Identification of Gaps

2.16 In the area of detection there is still gaps in our knowledge for the following:

- A rapid, simple test for the detection of VTEC organisms to be used for enforcement, monitoring and surveillance purposes, taking into account the sensitivity, specificity and speed of analysis.
- A method to detect all VTECs and not just *E. coli* O157.

2.17 In the area of differentiation, although sufficient work on the typing of VTEC has been undertaken, there remains the gap in the area of communication between researchers to ensure that validated methods are standardised both in diagnostic

and reference laboratories. There would also be benefits in researchers making use of a defined set of archived strains for research purposes to enable comparability between project results.

2.18 With respect to diagnosis of human disease, there is a gap in our knowledge for non-invasive diagnostic tests, e.g. salivary based tests.

3. MICROBIAL PHYSIOLOGY AND GENETICS

Introduction

3.1 The ACMSF(1) made a recommendation for Government-funded research on the nature and extent of acid resistance of VTEC. It was also recommended that the CMO's advice on the cooking of burgers should be reconsidered in the light of research into the relationship between the formulation and colour of cooked minced meat products, the colour of juices, the temperature achieved, and the survival of VTEC.

3.2 The WHO (3) recommended research to compare *E. coli* O157:H7 and other EHEC to common *E. coli*, for example, in respect of growth at 44°C, acid tolerance, heat tolerance, and survival during freezing. Recommendations also included investigating:

- the existence of viable, non-culturable forms of *E. coli* O157:H7 and other EHEC
- survival in acid or low a_w conditions
- the ability of EHEC to attach to surfaces and to form biofilms in the environment and on processing equipment
- survival/growth in whole packaged/cut vegetables under Modified Atmospheric Packaging
- interaction of EHEC with other organisms during fermentation of sausage, cheese, yoghurt, cereals, root crops, etc.
- whether current recommendations for heat treatment, such as 70°C, are adequate.

3.3 Twelve research projects fall within the category of microbial physiology and genetics. The research covers the areas of acid resistance, thermal death, survival in the environment, and toxin production by unculturable forms and under different growth conditions.

Current State of Publicly Funded Research

3.4 The acid resistance of *E. coli* O157 and other VTEC strains has been investigated. Although VTEC strains appear in general to be more acid resistant than non-VTEC strains, this is not an absolute finding, i.e. there is some overlap between the two groups. Exponential phase cells tended to be more sensitive to acidic conditions, and were more likely to demonstrate an acid habituation response, than stationary phase cells. Low concentrations of lactate or ethanol (or a combination of both) could overcome the acid resistance of VTEC, with this being linked to a collapse of the cytoplasmic pH to a low value. Exponential, habituated, and stationary phase cells were all sensitive to this treatment. It was noted that with VTECs there was the presence of an acid resistant 'tail' in kill curves (**DH, 245 & MAFF, FS1535**). A future project is to be funded which aims to develop molecular techniques to identify the actual protein and RNA components of acid tolerance and their role in generating inter-strain heterogeneity.

3.5 It has also been shown that exposure of *E. coli* O157 to low pH can result in cross-protection to other factors. Exposure to sublethal stress (pH 4.0) imparted a significant degree of protection, in comparison to a control culture at pH 7.0, for both heating at 56°C and 20% NaCl (**DANI, 9640**).

3.6 There have been a number of projects relating to the thermal death of *E. coli* O157 and other VTECs. There is a view that the thermal death kinetics of these organisms are not linear and that 'tailing' occurs. However, there is by no means a consensus on this subject, with views expressed that any tailing is an artefact of the experimental method used. A project is underway to investigate in detail the thermal death of pathogenic microorganisms in real foods. This is using *E. coli* O157 as a model organism and should help to clarify whether or not tailing does occur (**MAFF, FS3202**). Other projects have demonstrated that heating at 70°C for 2 minutes should be sufficient to inactivate *E. coli* O157 in beef and lamb products (**DH, 210 & 210B**).

3.7 A project to assess the effect of high pressure on the microbiological quality of foods has shown that survival of *E. coli* O157 in orange juice depends on the level of pressure applied, the treatment time and temperature and the pH (**DANI, 9702**). Certain strains have also been found to be unusually resistant to pressure. In addition, models have been developed to help determine appropriate processing conditions for poultry and dairy products.

3.8 Toxin production by VTECs has been investigated, one project having made an assessment of the extent of toxin production under specified growth conditions (**DH, 244**). It has been shown that toxin production can be enhanced using mitomycin C as an inducing agent and can improve the sensitivity of assays based on verocytotoxin (VT1 and VT2) production for detection of VTEC in faeces or foods. The toxins are produced in the intestine and absorbed into the blood where they affect vascular endothelial cells in target organs. The toxin consists of two subunits – A and B. The B subunit binds to the cell receptor on the cells of the lower intestine forming attaching and effacing lesions, whilst the A subunit enters the cell itself where it targets and stops the process of protein synthesis in ribosomes resulting in cell death. Other work has shown that some non-culturable cells of VTEC can synthesise and release toxins (**DH, 243**). Populations of VTEC cells in exponential phase were rendered non-culturable at 37°C by resuspending in phosphate-buffered saline at concentrations of $1-5 \times 10^8$ cells/ml in the presence of chloramphenicol. A vero-cell cytotoxicity assay was established and the researchers have shown that microcosms of non-culturable VTEC contain a cytotoxic factor which is neutralisable by the addition of specific verocytotoxin antisera. Preliminary data shows that *de novo* verocytotoxin production contributes to the cytotoxicity of non-culturable VTEC, rather than simply the presence of pre-existing cell associated verocytotoxin. The researchers have also shown that antibiotics, like mitomycin C, induce VT production and this finding has implications for the clinical treatment of VTEC patients. Another project has demonstrated that the expression of VT1 and VT2 toxins varies throughout the growth curve (**DH, 033**).

3.9 There is some evidence that *E. coli* O157 will survive for long periods in the environment. Results of one project indicates that the viable counts of the organism fell by only 1-2 logs in soil microcosms after 125 days (**DH, 033**). The survival of *E. coli* O157 in Scottish agricultural soils under a range of temperature and matric potential is currently being investigated (**SERAD, UAB/007/99**). A further study on the survival of *E. coli* O157 in Scottish agricultural soils and water systems also aims to determine safe practices for the disposal of potentially dangerous wastes to land which minimise the risk of contamination of water sources (**SERAD, SAC/204/98**).

3.10 The strain variation exhibited by *E. coli* O157 from human cases, food animals, food and the environment is being investigated. The project will seek to identify a series of simple, inexpensive tests to enable characterisation of different strains (**MAFF, FS3109**).

MSFFG Assessment and Identification of Gaps

3.11 Gaps in our knowledge relating to the physiology of VTEC (in terms of the effect of temperature, pH, and water activity) are currently being addressed through funded research. However, there are some areas where gaps remain:

- Survival of VTEC following heat treatment and the significance of the tailing effect of growth/kill curves
- Interaction of VTEC with other microorganisms in foods, e.g. in cheeses and fermented meats
- Survival and growth of VTEC in modified atmosphere packs
- The significance of survival of VTEC on raw vegetables as a vehicle of VTEC infection, and, in particular, sprouted seeds
- Effect of chlorination and other decontamination treatments on VTEC
- VTEC survival and adaptation to the environment, particularly on the farm and in composted materials
- Toxin production and its stability in foods, is it a food safety threat or not?

4. PATHOGENESIS

Introduction

4.1 The ACMSF (1) made a recommendation for Government-funded research on the effectiveness of clinical intervention in treating cases of VTEC infection and HUS. In particular, more needs to be known about:

- the efficacy of antibiotics in affecting carriage, spread of infection and outcome of infection
- the factors affecting the outcome of VTEC diarrhoeal illness, including the role of protective factors (age, sex, blood group) in progression to HUS
- the relationship between VTEC diversity in VT and adhesin production and clinical disease
- characterisation of the adhesins of VTEC strains, including the minority that do not produce the characteristic (attaching and effacing) lesions

4.2 A recommendation was also made for research on the nature and extent of acid resistance of VTEC.

4.3 The WHO (3) identified the following clinical data needs:

- evaluation of treatment modalities for the prevention of HUS following EHEC infection
- evaluation of management of HUS to prevent long-term complications following apparent recovery
- determination of the risks and benefits of antibiotic therapy in clinical cases of EHEC infection.

4.4 The WHO also recommended investigating which host factors are important in infectivity (susceptibility) and severity of disease and evaluate factors such as diet. Recommendations on the animal side included:

- defining animal colonisation: adhesion, growth and excretion in ruminants, shedding duration and frequency under various conditions, e.g. transportation and diet
- determining to what extent immune response occurs and the protective effects in animals
- determining the influence of growth promoters/antibiotics/therapy on rumen flora
- determining the adherence of EHEC to different surfaces, e.g. carcass and environment of slaughter house.

4.5 Ten research projects fall within the category of pathogenesis. The research covers the areas of risk factors for acquiring VTEC infection and factors which affect the clinical outcome of the disease, adhesion and colonisation of the organism, and the role of antimicrobials in the management of VTEC infections.

Current State of Publicly Funded Research

4.6 Several projects were identified in the area of immunopathogenesis of VTEC infection in humans. The relative roles and importance of bacterial, host and environmental factors in determining clinical outcome, including the development of haemolytic uraemic syndrome (HUS), are being addressed by a number of researchers. On the human side, work has recently started on investigating the local humoral immune response to *E. coli* O157 infection. Antibody production will be characterised in sporadic and outbreak cases and in subjects selected from the general population, at the mucosal level of the gastrointestinal tract. The potential role of passive immunisation in the treatment of patients with severe *E. coli* O157 disease will also be assessed. Initial development work on surface antigen preparation and ELISA development is complete and serum and gut lavage samples from patients and controls are currently being collected for antibody testing (**SODoH, K/MRS/50/C2624**).

4.7 Serum antibody production in patients infected with *E. coli* O157 is also being investigated. Researchers are considering the serological response to a number of putative virulence determinants and other VTEC components, including enterohaemolysin, flagella and intimin (**DH, 242**). Five secreted proteins, including intimin, are associated with the characteristic attaching and effacing (A/E) lesions of VTEC disease, and are chromosomally encoded on the Locus for Enterocyte Effacement (LEE). 62% of patients infected with *E. coli* O157 were found to produce antibodies to one or more of these proteins, suggesting that they were expressed during infection. Five of twelve sera from patients infected with non O157-VTEC also contained antibodies to one or more of these five secreted proteins. Preliminary results suggest that most patients with antibodies to O157 LPS also make antibodies to intimin. In contrast, VTEC enterohaemolysin and *E. coli* O157 flagellar antigens appear to be either poorly antigenic, or not expressed during infection. Researchers found that 70% of patients with serum antibodies to *E. coli* O157 LPS also made salivary antibodies to the O157 antigen. The significance of these results has yet to be established. Work being conducted elsewhere is also addressing the human immune response to LEE proteins, by examining antibody production in both serum and colostrum of individuals in the UK and elsewhere. (**BBSRC, 97/A2/D/03255**).

4.8 Host factors such as blood group and HLA type, and bacterial factors such as toxin production are being investigated using a combination of prospective and retrospective approaches. Clinical data has been collected on over 50 retrospective cases and the first phase of a prospective trial, which will investigate the use of competitive gut flora in reducing the impact of the disease, is nearing completion. Risk factors for acquiring the disease are also being considered as part of this work (**DH, 253**). Bacterial factors are also being investigated in a study examining the transfer and survival of the phage encoded verocytotoxin gene (VT2) between *E. coli* populations (**BBSRC/SERAD, BFP11345**).

4.9 A number of projects are being funded to investigate the mechanisms by which VTEC adheres to the gut epithelium and persists, both in human and bovine intestine. Both *in vitro* and *in vivo* approaches are being employed. The role of the proteins encoded on the LEE of VTEC is being evaluated using a range of genetic,

cell biology and immunohistochemical techniques (**BBSRC, 97/AD/D/03255**). One study has suggested that VTEC strains do not express fimbriae, but rather that outer membrane protein plays the major role in mediating VTEC adhesion to host cells *in vitro*. Further observations on VTEC adherence include clearly defined roles for the H7 flagellum and lipopolysaccharide, maximisation of adherence during the exponential phase of bacterial growth at pH 6, increased adherence under anaerobic conditions, and decreased adherence in conditions of low iron concentration (**DH, 248**). Other researchers are searching for novel adhesins of VTEC strains which do not produce the characteristic A/E lesions, by screening mutant libraries which they have prepared (**DH, 251**). Two hundred and fifty artificially generated mutants have been screened so far in *in vitro* adhesion assays, and ten mutants have been identified with reduced adherence which will be the subject of further genetic analysis (**DH, 251**). Early host/pathogen interactions have been assessed in human and cattle intestinal biopsies using *in vitro* organ culture and intestinal invasion quantified (**BBSRC, D10261/D10262**).

4.10 Relevant epithelial cell lines, such as human intestinal Caco 2 cells, have been used to help determine the molecular and cellular mechanisms involved in VTEC adhesion. Such studies have demonstrated alterations in epithelial cell membrane and cytoskeletal protein phosphorylation and gene expression during the early interactions of *E. coli* O157 with host cells (**SERAD, RR1/519/95**). Work is continuing on the influence of dietary nutrients and growth factors on these effects. The ability of VTEC to invade certain human epithelial cell lines has also been demonstrated. Another project has investigated attachment of *E. coli* O157 to human epithelial cells with the aim of identifying specific receptor systems and signalling cascades that are activated (**SERAD, RRI/519/95**). To date, alterations in membrane and cytoskeletal protein phosphorylation and gene expression have been observed. The influence of dietary nutrients and growth factors on these effects is being studied. Structural studies of VTEC adhesion to eukaryotic cells are also underway using nuclear magnetic resonance to characterise the cell-binding mechanism (**BBSRC/SERAD, BFP11329**).

4.11 A combination of organ culture and bovine surgical models will be used in a recently funded study to further characterise the interaction of VTEC with the intestinal mucosa (**BBSRC, D10262**). The process will be followed from its initial stages, using primary intestinal mucosal biopsies from humans and cattle, through to later events such as invasion, secretory and inflammatory responses using the ligated loop cattle model. The role of specific virulence factors will be assessed and signature-tagged mutagenesis will be used to identify novel virulence genes.

4.12 The establishment of a reproducible mouse model for VTEC infection, although not representing the pathogenesis of the human disease, has potential application in further pathogenesis studies and therapeutic evaluations (**DH, 033**). The model will allow the study of factors that contribute to the persistence of VTEC in the mouse intestine, such as adhesins and diet manipulations. The pathogenesis of *E. coli* O157 will be investigated in a new study using ovine and other animal models (**MAFF, OZ0706**).

4.13 Work in cattle suggests that large doses of *E. coli* O157 are necessary to produce infection, and result in intermittent excretion of low levels of the organism. Calves were found to excrete the organism for the longest periods, although immunity to infection did not develop (**MAFF, OZ0119**). Investigations using gnotobiotic calves have also provided evidence on the colonisation by VTEC of bovines (**MAFF, OZ0133**). Inoculated calves became heavily colonised with the infecting strain throughout the gastrointestinal tract, but the bacterium was not found in liver, kidney or skeletal muscle. The animals appeared well during colonisation and no histopathology was observed, including attaching and effacing lesions, *post mortem*, except for minor inflammatory changes in the intestinal tract. A recently funded project will use reverse transcriptase-PCR techniques to examine age related factors in the extent of colonisation of the alimentary tract of both adult cattle and calves born to carrier cows and any variance in virulence factor expression in these different categories of animal (**MAFF OZ0704**). A proteomic approach will be used in a new project to identify novel proteins and their encoding genes that are induced in the bovine gut in response to colonisation. A complementary project will identify the genes and their projects involved in the successful colonisation of cattle by *E. coli* O157. Another new project, which will start shortly, will address the question of whether VTEC strains with human pathological potential express factors which allow them to colonise and persist in the bovine host. Other work in cattle is aimed at determining the influence of VTEC on the bacterial community of bovine faeces and whether faecal shedding of VTEC is precipitated by a particular intestinal microbial community structure. (**SERAD, SCR/507/97**).

4.14 Research has been carried out into the effectiveness of clinical interventions in treating VTEC infection, particularly antibiotic therapy. There is currently little evidence on the role of antimicrobials in the management of VTEC-related infections. *In vitro* studies to address this have found that exposure of VTEC to sub-inhibitory concentrations (SMICs) of antibiotics will, in many cases, result in increased production of verocytotoxin, although significant inter-strain variation was evident. It was also observed that, in most cases, exposure to SMICs resulted in decreased adherence to human cell lines (**DH, 252**). *In vitro* studies have since suggested that the use of antibiotics can not be advocated, given the unpredictability of the organisms' response to such drugs and the increases in toxin production that are frequently seen (**DH, 243**).

4.15 Work has recently started on the development of detection tests for VTEC strains which could be considered to pose a greater hazard to human health, i.e. those strains best able to survive environmental stresses. Strain characteristics to be considered include survival on surfaces and tolerance to acid pH. Ultimately, these tests could find applications in the food industry (**MAFF, FS3109**).

MSFFG Assessment and Identification of Gaps

4.16 There are a lot of gaps in our understanding of the pathogenic mechanisms of VTEC. The main concern is that a lot of progress has been made in understanding the pathogenic mechanism of VTEC in humans, but this has not been put into context with the findings from gut ecology studies in animals where the organism has

a very different non-pathogenic relationship with its host. The following gaps in our knowledge have been identified:

- What is the attaching and colonisation mechanism in infected symptomatic calves and how does this compare to the findings in humans
- Why does VTEC adhere to some species and not others
- What is the role of virulence factors and, in particular, the role of the toxin in the disease process
- What is the significance of antibody production and the dynamics of the antibody reaction
- The significance of factors such as the human diet in relation to the virulence of the disease, e.g. the acquisition and role of iron in the disease process
- Resistance in humans and the role of asymptomatic carriers, particularly in relation to age and dose
- A role for an animal model to represent the human disease

• 5. EPIDEMIOLOGY

Introduction

5.1 The ACMSF (1) recommended that the Government, in association with the PHLS and Health Authorities, ensures that during outbreaks, case-control studies are undertaken to provide up-to-date knowledge about sources, routes of transmission, risk factors, and socio-economic costs associated with VTEC infection in the UK.

5.2 On the animal side, the ACMSF recommended research to:

- establish the incidence/prevalence of *E. coli* O157:H7 in UK cattle/cattle herds and other agricultural livestock
- improve understanding of the epidemiology of *E. coli* O157:H7 infections in agricultural livestock and identify the husbandry and other factors contributing to herd infection and control.

5.3 The Pennington Group (2) recommended research to:

- determine the prevalence/incidence of *E. coli* O157 in Scottish cattle and other animals, and the biology of its carriage
- help forecast its likely future incidence/prevalence

5.4 The WHO (3) recommended appropriate strategies for outbreak investigation in developing countries and sentinel site studies to better define the prevalence of EHEC infections in humans with various conditions.

5.5 On the animal side the WHO have recommended research on the sources and transmission of EHEC contamination of livestock (cattle, sheep, poultry, goats, horses, and pigs), so as to target intervention or develop prevention strategies regarding:

- survival in water, soil, and feed (including silage), manure/slurry
- transfer from mother to calf, faeces to hide, and human to animal
- decontamination of sewage and the use of slurry in agricultural practice

5.6 The WHO recommended research on the prevalence of EHEC in wild animals, insects, birds, fish and shellfish, pets, as well as livestock, and the herd-specific incidence of EHEC. Research on the prevalence/concentration of EHEC on carcasses during the entire slaughter process was also recommended.

5.7 Twelve research projects fall within the category of epidemiology. The research covers the areas of both human and animal epidemiology with respect to VTEC, and includes fully integrated studies looking at the whole food chain.

Current State of Publicly Funded Research

5.8 A major study, the Infectious Intestinal Disease (IID) Study in England, has been funded in order to provide information about the incidence, sources, routes of transmission, risk factors and socio-economic costs of infectious intestinal disease, including VTEC (**DH, 177 and 178**). Published data from the study indicate that IID occurs in 1 in 5 people each year, of whom 1 in 6 presents to a general practitioner. This means that nine and a half million cases of IID occur annually, of which one and a half million present to their GPs. For every 1,000 cases of IID in the community, 160 presented to their GP, 45 had a stool sent routinely for microbiological examination, 10 had a positive result, and 7 were reported to the Public Health Laboratory Service Communicable Disease Surveillance Centre (PHLS CDSC). The ratio varies according to the microorganism. Approximately 3 cases of salmonellosis occur in the community for every 1 report to CDSC, whereas as many as 1500 or more cases of small round structured virus infection may occur for every 1 reported to PHLS CDSC. *E. coli* O157 was isolated from only 3 cases from the GP component of the study (0.1%) and not in cases from the community who did not present to a GP, nor from asymptomatic controls.

5.9 A considerable amount of work has been funded to investigate the epidemiology of *E. coli* O157 disease in Scotland. A 1991 study used questionnaires to collect evidence on transmission routes and host risk factors (**SODoH, K/MRS/50/C1820**). The researchers found an unexpectedly high proportion of cases had been exposed to environmental factors, such as farm animals or gardening, or had had household water supply problems prior to becoming ill. Further research has been funded in Scotland to evaluate the relative importance of environmental risk factors in sporadic cases of *E. coli* O157 infections, in particular whether disruption to domestic water supply is significant (**DH, 247**). Patients are being followed for extended periods of time, to examine the rates and nature of long term sequelae of infection with the organism. Data is still being collected, and will be fully analysed when complete. The dispersal of *E. coli* O157 in Scottish agricultural soils and the potential for contamination of private water supplies is currently being investigated (**SERAD, UAB/007/99**).

5.10 Patients who contracted *E. coli* O157 during the 1996 Central Scotland outbreak are being followed up for up to three years in order to determine how the illness has affected their continuing quality of life, including the development of chronic sequelae such as renal abnormalities and gastrointestinal problems (**SODoH, K/MRS/50/C2671**). The predictive value of clinical features such as fever and oliguria, laboratory parameters such as leucocyte count, and/or complications such as HUS on the development of such sequelae will be determined.

5.11 Molecular fingerprinting techniques have been used to evaluate the evolution of VTEC in Scotland, its population structure, sources and routes of transmission. This study has paid particular attention to determining the significance of non-O157 VTEC in enteric disease (**SODoH, K/MRS/50/C2376**). The researchers found that PFGE, PCR and DNA sequencing techniques allowed definitive characterisation of *E. coli* O157 isolates. The same genotypes of *E. coli* O157 were identified in food animals and human infection, suggesting an infective link. The techniques

developed in this study, particularly PFGE, have provided valuable evidence of transmission during the real-time investigation of O157 outbreaks. Data generated in this study now comprise a database of genotypes to which new isolates can be compared, and through which recurrent clones of the organism which cause significant human disease have been recognised. The researchers found that the prevalence of O157 VTEC and non-O157 VTEC strains is similar within Scotland, and that the diverse group of non-O157 VTEC contained significant human pathogens.

5.12 Conventional strain typing techniques had suggested that *E. coli* O157 isolated from three Scottish outbreaks represented a Scottish “superclone” of the organism. Further independent studies, conducted in 1997 at two *E. coli* O157 reference laboratories, used a combination of molecular fingerprinting techniques to demonstrate that this was not in fact the case. The isolates from the three outbreaks could be distinguished using these more sophisticated techniques (**SERAD, LEP/001/97, UAB/004/97**).

5.13 An epidemiological study in Scotland is considering the distribution of *E. coli* O157 infections, and its more severe disease manifestations, amongst patients expressing particular blood group phenotypes (**SODoH, K/MRS/50/C2601**). The researchers are particularly concerned with differences in cell adhesion, haemolysin and verotoxin activity in groups of patients. The study will assess if cell adhesion in the elderly and children, both higher risk groups, is different to that in young adults, when compared within the same blood group phenotype. A separate Scottish study is also considering the role of blood group phenotype in the presentation of disease (**DH, 253**). These studies are still collecting data and no results are yet available.

5.14 A national study in England is also investigating the relative importance of risk factors for the acquisition of infection with *E. coli* O157 (**DH, 241**). The properties of implicated vehicles which may increase their probability of causing infection, e.g. rare beef dishes, will be identified. This study will include considerations of the importance of secondary spread of infections within households. The data collection phase has been completed for this work, and analysis is underway.

5.15 Valuable evidence has been provided on the incidence of VTEC O157 in Wales, the epidemiology of the disease and characteristics of cases and infection sources. Overall, VTEC O157 remained a rare disease in Wales over the period examined; the average annual incidence of infection was 1.45 per 100,000 resident population in 1994 and 1995, and only one outbreak was identified in 1995 (**DH, 145**). Cases peaked during the summer months and had a wide geographic distribution, with children under five years comprising the highest number of cases. Of the sporadic cases, a large proportion (60%) reported haemorrhagic colitis, 6% (mostly children under five years old) developed HUS and 6% were asymptomatic. High risk groups for the development of haemorrhagic colitis included the elderly and those patients taking antibiotics. Sources of infection included direct contact with cattle, other infected individuals and particular foodstuffs, including inadequately-cooked beefburgers and cooked sliced meats (presumably via cross contamination). A subsequent study in 1997 found that Wales saw an increase in the incidence of VTEC haemorrhagic colitis, the causes of which have yet to be

determined (**DH, 254**). The researchers identified that living on, or visiting, farms could be risk factors in acquiring the infection. This aspect was investigated in some detail. VTEC excretion and seroprevalence of anti-O157 LPS antibodies were followed in a cohort of farmers; VTEC genetic material was detected in 6.3% of faecal samples from participating farmers, and all faecal isolates were of serogroups other than O157. Risk factors for VTEC excretion amongst participating farmers included having a non-mains water supply and contact with sheep. 1.7% of serum samples contained anti-O157 antibodies, from individuals who were all involved in the application of slurry to land but who remained apparently healthy.

5.16 A more integrated approach to the epidemiology of VTEC has been adopted by researchers in Sheffield. Although a local study, this work has implications for the national situation and has received considerable media attention. Initial work considered the prevalence and seasonal distribution of VTEC O157 in food animals, selected foodstuffs and human faecal samples (**DH, 182**). The study made a number of significant findings, in particular that the overall prevalence of the organism in cattle at slaughter was higher at 15.7% than has been suggested by previous studies. This prevalence was seasonal, with monthly rates varying between 6% and 37%. *E. coli* O157 was found in 2.2% of sheep examined and preliminary data suggests that, although prevalence of the organism in sheep was lower than in cattle, lamb products may be more frequently contaminated than beef products. Almost all the strains isolated from sheep and cattle were representative of strains causing infections in man. In contrast, strains isolated from pigs were of types unlikely to be a source of human infection. A follow-up study is in progress looking in more detail at these findings, particularly at the relationship between strains isolated from sheep and sheep products, and cattle and beef products (**DH, 182B**). In addition, the prevalence of antimicrobial resistance amongst *E. coli* O157 throughout the food chain will be determined. Initial results from this extended study are currently being examined, and molecular typing of a selection of strains from the studies is in progress.

5.17 Research in Scottish cattle is aimed at determining the prevalence of farms with cattle carrying VTEC O157 and the proportion of animals on those farms which are positive for the organism (**SERAD, SAC/168/97 and UAB/006/97**). This information will be correlated with farm management practices. These studies are ongoing and progress is yet to be reported. A national study in cattle, during 1994-1997, found clusters of infected animals within herds; the highest prevalence was amongst young calves without symptoms (**MAFF, OZ0119**). A longitudinal study of faecal excretion of *E. coli* O157 in cattle has recently started (**MAFF, OZ0138**). Another recently funded project will investigate the spatial and temporal patterns of transmission and dissemination of foodborne pathogens, including *E. coli* O157, from the farm environment to the abattoir (**VF0201**).

MSFFG Assessment and Identification of Gaps

5.18 A lot of good epidemiological studies have been funded on both the human and animal side. The research has identified three main routes of transmission:

1. Foodborne transmission
2. Person-to-person spread, and
3. Environmental spread

We currently do not know the relative magnitude of these three pathways, e.g. is the foodborne route the most important? Research in this area will lead to a better understanding of the relative risk factors for disease. Additional gaps in our knowledge were identified as follows:

- Do particular groups (e.g. farm workers) have an increased risk of VTEC infection within populations
- What are the causes of sporadic infection and what is the significance of non-foodborne infection (e.g. water)
- The reasons for geographical variation in the incidence of infection
- The role of seasonality and travel
- The epidemiology of non-O157 VTECs

5.19 On the animal side, research is well underway to determine the prevalence of carriage in cattle. However, there remains a gap in our knowledge on the prevalence of excretion and determining the factors that control excretion. Additional gaps were identified as follows:

- Consideration of the routes of transmission to food animals and environmental sources of the organism (e.g. from water)
- The prevalence of VTEC in wild animals, poultry and pets

6. SURVEILLANCE

Introduction

6.1 The ACMSF (1) made a recommendation that the Government ensures that relevant clinical groups set up national surveillance studies of HC, HUS and TTP in all age groups. On the food side, the ACMSF recommended research on the prevalence of O157 VTEC in raw meats, raw cows' milk, cream made from raw cows' milk, and raw milk cheeses;

6.2 The WHO (3) recommended population-based surveillance for HUS. Ideally, HUS surveillance should be coupled with microbiological and serological studies to determine the serotypes of HUS-causing strains. An evaluation of the role of serodiagnosis in general surveillance and in outbreak investigation was also recommended. On the food side research on the prevalence and survival of EHEC on herbs and spices and fruit juices was recommended.

6.3 Seventeen research projects fall within the category of surveillance. The research covers the areas of food surveillance and surveillance of human illness associated with VTEC infection.

Current State of Publicly Funded Surveillance

6.4 A number of groups in England, Wales and Scotland have considered the surveillance of VTEC within the human population. Certain of these studies have been considered under "pathogenesis", as they are considering host risk factors and response to infection. Separately, work has been undertaken to provide enhanced surveillance of VTEC in the human population, foodstuffs and the environment, in support of laboratories throughout the UK (**DH, 104**). Serotyping, phagetyping and toxin subtyping have been used to examine both sporadic and outbreak cases. The majority of VTEC strains identified belonged to serogroup O157 (98% in 1990 and 1991, >99% in 1992 and 1994, 88% in 1993). Phage type (PT) 2 and PT49 predominated in 1990-1993, with PT2/VT2 predominating in 1992-1993, particularly during outbreaks. VTEC was detected in various foods, including sausages, raw beef products and beefburgers, unpasteurised milk and cattle faeces, but not in chickens. Non-O157 VTEC strains were also demonstrated in foods.

6.5 Two projects investigating the importance of risk factors in the acquisition of *E. coli* O157 infection (**DH, 241 and DH, 247**) have been discussed under the section heading "Epidemiology". The former project, a case control study of *E. coli* O157 infection in England, will identify risk factors for HUS and the findings will be linked into the British Paediatric Surveillance Unit data.

6.6 Work has been undertaken throughout the UK to examine the prevalence of *E. coli* O157 in abattoirs. In Northern Ireland during 1995-1998, sampling of faeces immediately *post mortem* showed two animals, out of 220 tested (<1%), to be carriers of *E. coli* O157 (**DANI, 9723**). Hide pulling was identified as the principal

critical control point in determining the microbiological quality of beef carcasses in the 7 abattoirs examined. Forty three small and large domestic abattoirs, including those for export, were surveyed in England and South Wales during 1992, by determining the intestinal carriage of *E. coli* O157 in beef cattle at slaughter (**DH, 124**). Fourteen of 211 samples (7%), a higher rate than in N. Ireland, were positive for *E. coli* O157, with 2 of the isolated strains testing positive for VT. The positive samples contained O157 VTEC at very low frequency (<0.2%). A national abattoir survey, conducted within the period 1994-1997, found *E. coli* O157 on 0.47% of carcasses and within the faeces of 0.59% of pre-slaughter cattle. In Scotland, microbiological assessment of bovine and lamb carcasses forms part of a wider study aimed at minimising pathogen contamination of carcasses, including VTEC. A local study carried out in Sheffield (**DH, 182**) found the prevalence of the organism in cattle at slaughter to be higher than has been suggested by previous studies (a prevalence of 15.7%). This prevalence was seasonal, with monthly rates varying between 6% and 37%. *E. coli* O157 was found in 2.2% of sheep examined and preliminary data suggests that, although prevalence of the organism in sheep was lower than in cattle, lamb products may be more frequently contaminated than beef products (see epidemiology section for further details).

6.7 Surveillance of VTEC in foods has centred on raw meats and raw milk. A study in Southeast Scotland, initiated in 1997, is examining raw meats, including lamb, raw cows' milk and raw milk cheese for the presence of VTEC O157 (**DH, 250**). Meat and cheese samples are being obtained from retail outlets, and milk samples from farms. Sample analysis continues but, so far, the researchers have reported finding two samples of beefburger containing VTEC O157, of which both were PT32. Raw meat surveillance is included in the study in Sheffield (**DH, 182**), which contains elements of food and food animal surveys, and has produced some interesting findings on the prevalence of the organism in that geographical area. An extensive survey of the microbiological safety of raw cows' milk on retail sale has been undertaken in England and Wales (**DH, 171**). Samples were collected over a 12-month period during 1995-1996 and analysed for a range of criteria, including the presence of *E. coli* O157. Of the samples confirmed as raw milk, 16% had total viable counts and 24.5% had coliform counts which failed the standards specified in the Dairy Products (Hygiene) Regulations 1995, although *E. coli* O157 was not detected in any of the samples examined.

6.8 Certain projects aimed at developing improved methods for detecting VTEC in foods contain elements of survey methodology in the testing of the methods. These projects are considered under the section entitled Detection, Differentiation and Diagnosis.

6.9 A project started in 1999 is addressing the prevalence of foodborne pathogens, particularly *E. coli* O157 in cattle and sheep slaughtered for human consumption and results should be available later this year. (**MAFF, FSZ2500**). A study is also in progress to determine the prevalence of foodborne pathogens in pigs at slaughter and these results will also be available towards the end of 2000. (**MAFF, FT9119**).

6.10 A continuous study is being undertaken to examine those animal premises which have been implicated in human outbreaks of *E. coli* O157 in England and Wales (**MAFF, FSZ2100**). The main objective is to determine whether excretion from implicated animal groups is occurring on the day of visit. Isolates are typed and general hygiene advice is also given.

MSFFG Assessment and Identification of Gaps

6.11 The identification of gaps and overlaps in this area is a matter for the Microbiological Food Surveillance Group, the Epidemiology of Foodborne Infections Group and the Veterinary Surveillance Group set up under the CVO. Projects on surveillance will continue to be listed under the MSFFG system, but the work will not be discussed by the Funders Group.

7. RISK ANALYSIS

Introduction

7.1 The WHO (3) recommended research to evaluate the effectiveness of preventive strategies such as the implementation of HACCP. With respect to fresh plant produce and related products, the WHO recommended a thorough risk assessment for EHEC (and other) infections arising from seeds/beans for sprouting.

Current State of Publicly Funded Research

7.2 A new project is being funded that aims to develop a systems analysis methodology for *E. coli* O157:H7 in sheep and cattle production systems and to document this methodology. The project hopes to develop a framework of the life cycle of *E. coli* O157:H7 in cattle and sheep and both identify and extract the data required to quantify infection and contamination risks. This will also be documented. The main objective of the project is to quantify the probabilities associated with infection hazards and to generate a probability network model that can be applied to identify critical control points and the subsequent recommendations.

MSFFG Assessment and Identification of Gaps

7.3 The Group noted that appropriate risk analysis can only be carried out where the appropriate evidence/data is available and this will help focus future research priorities. The following gaps in our knowledge have been identified:

- Risk assessments to assess the risk of VTEC in food relative to other sources (e.g. the environment, person to person contact) i.e. a critical pathway analysis
- Consideration of how consumers perceive the risk of VTEC from various sources, e.g. from food, on the farm, etc., and how to address risk communication on this basis
- An investigation of the effectiveness of HACCP as a risk management tool.

8. REDUCTION AND ELIMINATION

Introduction

8.1 The ACMSF (1) made a recommendation for Government-funded research on the effectiveness of processing aids, such as carcass washes, in further reducing the microbiological load on carcasses. Research on the effect of sanitisers/disinfectants on the survival of VTEC was also recommended. On the food side, the ACMSF recommended research into the relationship between the formulation and colour of cooked minced meat products, the colour of juices, the temperature achieved during cooking and the survival of VTEC.

8.2 The WHO (3) recommended research on the development of decontamination methods for seeds and fermented products. On the animal side the WHO recommended an evaluation of methods to reduce the presence of EHEC in farm animals (e.g. the use of colicin-producing organisms, competitive exclusion, VTEC-specific phage) and the determination of the susceptibility of EHEC to destruction/washing on different types of animals (e.g. acid washes, pasteurisation, vacuum pasteurisation).

8.3 Nine research projects fall within the category of reduction and elimination. The research covers the areas of the effectiveness of disinfectants in eliminating the pathogen, the effectiveness of the cooking process, the effect of high pressure processing, and the decontamination of fruit and vegetables.

Current State of Publicly Funded Research

8.4 Four projects have investigated cooking and the degree of heat treatment required to destroy *E. coli* O157. It was found that heating to 70°C for 2 minutes was adequate to destroy the bacteria in both beef and lamb products. However, research showed that the colour of the meat was not an adequate index for the estimation that meats had been heated to this level. A more wider reaching project is investigating whether current methods for predicting the thermal death of bacteria and spores requires re-evaluation. A literature review of previous work on thermal inactivation is currently underway and is assessing the major causes and extent of tailing during thermal death (**DH, 210; MAFF, FS1538, FS1539 & FS3202**). *E. coli* O157 has been shown by previous research to be sensitive to irradiation.

8.5 A comprehensive review of methods of meat decontamination was carried out in the mid-1990s as part of the MAFF Fellowship in Food Process Engineering at the University of Bristol. The review included evaluation of the potential application of existing, new, and novel processes and also defined the most promising areas for further research.

8.6 A project to investigate the efficacy of disinfectants used in the food industry against *E. coli* O157 and other pathogens has recently started (**MAFF, FS3206**). It

aims to use both existing and newly developed methods including suspension tests, surface film tests and real sample testing.

8.7 Hazards associated with the contamination of fruit and vegetables by bacterial pathogens are being addressed in a project which looks to demonstrate the feasibility two novel techniques for disinfection (**MAFF, FS3208**). The techniques to be assessed are ultrasound and a photodynamic system. Six model foods and three species of bacteria, including *E. coli* O157, will be used.

8.8 A project to assess the effect of high pressure on the microbiological quality of foods has shown that survival of *E. coli* O157 in orange juice depends on the level of pressure applied, the treatment time and temperature and the pH (**DANI, 9702**). Certain strains have also been found to be unusually resistant to pressure. In addition, models have been developed to help determine appropriate processing conditions for poultry and dairy products.

8.9 The survival of VTEC in the environment has been investigated (**DH, 033**). The findings indicate that after 125 days, viable counts of *E. coli* O157 in soil only fell by 1 - 2 logs, indicating that the bacteria can survive for extended periods.

8.10 Work is currently being funded under the LINK initiative into the effectiveness of steam condensation for carcass decontamination and is funded under the Advanced and Hygienic Food Manufacturing LINK Programme. There is also a project (**MAFF, FS1043**) which is investigating decontamination of cuts of meat using pressure and organic acids. A second project concerned with meat hygiene aims to identify the main points of entry of pathogens, including *E. coli* O157, to bovine meat and lamb in slaughterhouses and to investigate methods of minimising such contamination (**SERAD, SAC/125/96**). A recording system has been established to evaluate the cleanliness of animals at the point of slaughter and microbiological protocols are being refined to attempt to correlate cleanliness scores with bacterial contamination of carcasses. A project in Northern Ireland has shown that, in terms of microbial quality of beef carcasses, hide pulling was the principal critical control point (CCP) (**DANI, 9723**).

8.11 Work is currently underway to control *E. coli* O157 in the ruminant gut and in the farm environment (**MAFF, OZ0702**). The recently funded project will investigate methods for dietary control of the organism in the ruminant gut, the use of probiotic bacteria to suppress growth of *E. coli* O157, as well as the effectiveness of acid/ethanol treatments on reduction of the organism. An alternative approach to control of *E. coli* O157 in cattle is being addressed in a recently funded project which utilises a novel plant antibody delivery system to immunise livestock against the organism (**MAFF, OZ0703**). Another study is using molecular techniques to inhibit the expression of virulence genes in infected cattle (**BBSRC/SERAD, BFP11349**). The results of these studies are not yet available.

MSFFG Assessment and Identification of Gaps

8.12 The following gaps in our knowledge have been identified:

- Survival of non-culturable VTEC and other stressed forms following heat treatment
- Simple and practical ways of measuring the internal temperature of burgers
- A review of the effectiveness of carcass washing on the removal of VTEC
- A review of HACCP – to avoid VTEC contamination of the carcass

9. MICROBIAL ANTIBIOTIC RESISTANCE

Introduction

9.1 The ACMSF considered the very complex issue of microbial antibiotic resistance in relation to food safety and made a wide range of recommendations for research in its recently published report (4). The ACMSF regarded two areas as particularly important:

- Work is needed on the chain of events which can lead to antibiotic-resistant microorganisms arising from farming practices, being transmitted through food chain pathways, and causing human infection
- Research is very much needed on possible exposure of general, animal and food microbial flora to resistance, with the accompanying risk of the establishment of a reservoir for the transfer of such resistance to humans

9.2 To date, only one project has been placed in this category. It is possible that the number of projects in this category will increase in the future as funders commission work to address recommendations following publication of the ACMSF Report.

Current State of Publicly Funded Research

9.3 A component of a local study investigating the prevalence of VTEC O157 in the Sheffield area (**DH, 182B**) is monitoring possible emergence of microbial antibiotic resistance in *E. coli* O157. Recent *E. coli* O157 and non-O157 isolates from cattle, sheep, food and humans have been examined, as well as *E. coli* O157 isolates from the early 1990s (mostly human isolates). Although the data has not yet been fully analysed, it does appear that the proportion of resistant *E. coli* O157 is more frequent in recent isolates than in the older isolates. A higher proportion of resistant isolates also occurred in those which were non-O157 *E. coli*.

9.4 Next year will see the start of several projects on antimicrobial resistance that will include studies of *E. coli*. These projects are in the later stages of being approved and a definitive list is not yet available.

9.5 Further research in this area may be funded in response to the recommendations of the ACMSF report on Microbial Antibiotic Resistance. This area will be assessed at a future date.

10. OTHER

Introduction

10.1 The ACMSF (1) made a recommendation for Government-funded research on the socio-economic costs associated with VTEC infection in the UK.

10.2 The WHO (3) recommended research to determine the economic impact of *E. coli* O157:H7 and other EHEC infections. A recommendation was also made for an assessment of various communication modalities for their effectiveness with different groups in an outbreak setting.

10.3 One research project falls within this “other” category and covers the area of the socio-economic impact of VTEC infection. In addition, the socio-economic costs of infectious intestinal disease, including VTEC, has been investigated as part of a major study, the Infectious Intestinal Disease (IID) Study in England (**DH, 177 and 178**). A report on the IID Study is expected shortly.

Current State of Publicly Funded Research

10.4 The economic and social impacts of an outbreak of *E. coli* O157 infection, which occurred in Scotland in 1994 and was linked to pasteurised milk, was assessed in a recent study (**DH, 219**). The researchers were able to estimate the overall financial cost of the outbreak and made a number of recommendations relating to its management, including the need for improved communication between the various healthcare agencies and the public, and the need for improved ascertainment. They found that the costs of illness were much higher in patients who developed HUS or thrombocytopenic purpura. The researchers state that most savings would accrue from preventing the outbreak in the first place, by considering each stage of the food chain.

MSFFG Assessment and Identification of Gaps

10.5 Projects falling into this area of research, e.g. the socio-economic costs of foodpoisoning outbreaks, provide information on the impact of food poisoning on society. Future funded work on risk communication will be included in this area.

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3. The World Health Organization. Prevention and control of enterohaemorrhagic *Escherichia coli* (EHEC) infections. (1997); Food Safety Unit, World Health Organization, Geneva, Switzerland
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APPENDIX

A 1.1 Glossary

ADHESIN

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

a_w

Water activity/ionic product of water. A measure of the amount of solute present and consequently the amount of water available to a microorganism.

CaCO 2 CELLS

Human colon adenocarcinoma cells. This is a human intestinal epithelial cell line which differentiates after forming a confluent monolayer to produce a single, polarised layer of cells with typical brush border microvilli on the apical surface.

eaeA

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

EHEC

Enterohaemorrhagic *Escherichia coli*.

ENTEROHAEMOLYSIN

Causes lysis of red blood cells

HC

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

HEp-2 CELLS

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

HUS

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

INTIMIN

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

LEE

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

LPS

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

MITOMYCIN C

An antimicrobial agent originating from *Streptomyces* spp. that prevents DNA replication by crosslinking the complementary strands of the double helix.

O157 VTEC

Verocytotoxin-producing *Escherichia coli* of serogroup O157.

PCR

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

PFGE

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

RFLP

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

RT-PCR

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

Sandwich ELISA

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

SDS-PAGE

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

TTP

Thrombotic Thrombocytopenic Purpura. A clinical condition which can result from infection with VTEC and is characterised by a reduction in the number of blood platelets, inducing bleeding into the skin and spontaneous bruising.

VTEC

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

A 1.2 Research and Development projects considered in the discussion paper.

Project Code	Project Title	Start Date	End Date	Funder
BFP11329	Structural studies of host-cell/pathogen adhesion in enteric bacterial infection	06/01/99	05/31/02	BBSRC /SERAD
BFP11335	<i>E. coli</i> diversity in ruminants and its effect on the carriage of enterohaemorrhagic <i>E. coli</i>	03/01/99	02/28/02	BBSRC /SERAD
BFP11345	The role of bacteriophage in maintenance and transfer of VT genes in gastrointestinal <i>Escherichia coli</i> populations	04/01/99	03/31/02	BBSRC /SERAD
BFP11349	Inhibition of virulence gene expression by targeted redirection of RNase P	04/01/99	03/31/02	BBSRC /SERAD
D10261 & D10262	Characterisation of the interaction of enterohaemorrhagic <i>Escherichia coli</i> with intestinal mucosae	10/01/98	09/30/02	BBSRC
3258371	Microbial adaptation to environment and survival in the food chain	04/01/98	03/31/01	BBSRC
97/A2/D/03255	Pathogenicity of enterohaemorrhagic <i>E. coli</i> (studentship)	10/02/97	10/01/00	BBSRC
9640	An investigation of the phenomenon of cross-protection in verocytotoxigenic <i>E. coli</i> O157	05/01/97	04/30/00	DANI
9702	Effects of high pressure on the microbiological quality of foods	11/01/97	10/31/00	DANI
9723	Microbiological quality of beef carcasses in Northern Ireland - A baseline study	01/01/97	12/31/98	DANI
O33 with O33a	Survival and physiology studies of verotoxigenic <i>Escherichia coli</i> O157	04/01/94	03/31/97	DH
O33a with O33	Detection of <i>E. coli</i> serotype O157 using bacteriophage - based systems (linked with O33)	12/01/91	03/31/96	DH
104	Surveillance of verotoxin-producing <i>E. coli</i> in relation to human disease and the sources and vehicles of infection	03/01/90	02/28/92	DH
124	Survey of <i>E. coli</i> O157 in abattoirs	06/15/92	07/14/92	DH
145	Incidence and sources of <i>E. coli</i> O157 associated illness in Wales	09/13/93	09/12/96	DH
171	Surveillance of the microbiological safety of raw cows milk on retail sale; Subtyping and archiving	05/01/95	09/30/96	DH
182	Epidemiology of VTEC infections in the Sheffield area	04/01/97	03/31/99	DH
195	Detection of specific mRNA as an index of viability in stressed foodborne micro organisms	04/08/94	04/07/98	DH
210	Assessment of the degree of the heat treatment necessary to destroy <i>E. coli</i> O157 in meat	01/08/95	31/07/97	DH
210B	Assessment of the degree of the heat treatment necessary to destroy <i>E. coli</i> O157 in meat	10/01/97	07/31/98	DH
219	An economic assessment of the impact of an outbreak of <i>E. coli</i> O157 infection	01/01/95	06/30/95	DH

241	(VI) The Public Health Laboratory Service case-control study of <i>Escherichia coli</i> infection in England	03/01/96	09/30/97	DH
242	Serological tests for evidence of infection with VTEC based on serum antibodies to enterohaemolysin and adhesins	06/01/96	05/31/99	DH
243	Toxin production by non-culturable verotoxigenic <i>E. coli</i>	04/01/96	03/31/99	DH
243B	Toxin production by non-culturable verotoxigenic <i>E. coli</i>	04/01/99	10/31/99	DH
244	Development and evaluation of solid media to identify VTEC and to detect verocytotoxin in clinical and food samples by a novel method producing a different colour response to both the toxin antigen and its biological activity	06/10/96	06/09/99	DH
245	Acid tolerance and pH homeostasis of VTEC	03/18/96	03/17/99	DH
246	Development of monoclonal antibody based sandwich ELISA(s) for the rapid detection of verocytotoxin producing <i>E. coli</i> (VTEC) strains	05/01/96	09/30/99	DH
247	(I) Long-term sequelae of identification with <i>E. coli</i> O157: a national case register of laboratory identified cases, (II) A case-controlled study of sporadic cases of <i>E. coli</i> O157 infection in Scotland	10/01/96	03/31/99	DH
248	Verocytotoxin producing <i>E. coli</i> : Characterisations of adhesins	03/01/96	02/28/99	DH
250	A survey of the prevalence of <i>E. coli</i> O157 in raw meats, raw cow's milk and raw milk cheeses in SE Scotland	01/01/97	07/31/98	DH
251	Identification of novel adhesins of VTEC strains lacking the eae determinant	01/01/97	12/31/99	DH
252	The effect of anti-microbial agents on the pathogenesis of verocytotoxin producing <i>E. coli</i> (VTEC) related infection	06/03/96	12/02/97	DH
253	<i>E. coli</i> O157 infection and the role of the host, the bacteria and the environment in determining the outcome of the disease process	07/01/96	06/30/99	DH
254	Verotoxigenic <i>E. coli</i> including serotype O157: the population burden and the role of zoonotic spread	04/01/96	12/31/97	DH
FS1043	Decontamination using condensation under modified pressure and organic acids	01/06/96	31/12/98	MAFF
FS1231	Molecular and immunological approaches to the separation, concentration and detection of VTEC including <i>E. coli</i> O157	01/01/96	12/31/97	MAFF
FS1238	Development of improved methods for typing of verocytotoxin producing <i>E. coli</i> (VTEC) O157 and other serogroups, from food	09/04/95	09/04/98	MAFF
FS1260	Preparation of 'validation samples' for use in the evaluation of microbiological methods	04/01/98	02/01/00	MAFF
FS1531	Mechanisms of microbial resistance to high hydrostatic pressure	04/01/96	07/31/99	MAFF
FS1535	Mechanism of acid resistance of enterohaemorrhagic <i>E. coli</i> O157	04/01/96	10/02/98	MAFF

FS1538	Assessment of the survival of vegetative pathogens during the cooking of burgers	09/01/96	11/30/96	MAFF
FS1539	Survival and recovery of verocytotoxigenic <i>E. coli</i> and other organisms heated in ground beef	08/19/96	12/18/96	MAFF
FS3109	Development and study of tests to differentiate between tolerant and sensitive isolates of Salmonella and <i>E. coli</i>	04/01/98	03/31/01	MAFF
FS3202	Thermal death of pathogenic micro-organisms in real foods	05/01/97	04/30/00	MAFF
FS3206	Efficacy testing of disinfectants used in the food industry against a range of pathogens including <i>E. coli</i> O157	05/01/98	10/31/99	MAFF
FS3208	Novel methods for cleaning and decontaminating raw vegetables and fruit	04/01/98	03/31/00	MAFF
FSZ2100	Surveillance of animal premises associated with human outbreaks of VTEC O157	Continuou s		MAFF
FSZ2500	Survey to measure the prevalence of excretion of foodborne pathogens by cattle and sheep presented to abattoirs in GB for slaughter for human consumption	1999	2000	MAFF
FT9119	Survey to measure the prevalence of foodborne zoonotic organisms in pigs at slaughter	1999	2000	MAFF
OZ0119	Epidemiological studies of <i>E. coli</i> O157 infection in cattle	04/01/94	03/31/97	MAFF
OZ0133	<i>E. coli</i> O157:H7 colonisation and persistence in cattle	04/01/97	03/31/00	MAFF
OZ0138	A longitudinal study of faecal excretion of VTEC O157 in cattle	10/01/98	09/30/02	MAFF
OZ0141	Evaluation of the present status of <i>E. coli</i> O157 (and other VTEC) detection and diagnosis	07/06/98	04/05/99	MAFF
OZ0306	Hygienic design and manufacturing practices for pathogen-free poultry feed production	04/01/96	03/31/99	MAFF
OZ0702	Control of <i>Escherichia coli</i> O157:H7 in the ruminant gut and in the farm environment	04/01/99	03/31/02	MAFF
OZ0703	Plant antibody delivery of passive immunisation against <i>E. coli</i> O157:H7 a novel means of control in the animal	04/01/99	12/31/02	MAFF
OZ0704	Quantification of <i>E. coli</i> O157:H7 virulence factors in vivo using real-time RT-PCR and in situ RT-PCR	06/01/99	05/31/02	MAFF
OZ0706	EHEC O157 Pathogenesis: Ovine and Animal Model Studies	04/01/99	03/31/02	MAFF
RRI/519/95	Molecular and cellular mechanisms of bacterial attachment and invasion	04/01/95	03/31/99	SERAD
SAC/125/96	Inter-relationships between livestock condition, slaughterhouse practices and the microbiological safety of carcasses	04/01/96	10/31/00	SERAD
SAC/168/97	Determination of the prevalence of <i>E. coli</i> O157:H7 in Scottish beef cattle	04/01/98	06/30/00	SERAD
SAC/204/98	Survival of <i>E. coli</i> O157 in Scottish soil and private water supplies	01/14/99	01/13/02	SERAD
SCR/507/97	Faecal microbial community structure in VTEC and non VTEC carrying animals	02/01/98	12/31/98	SERAD
LEP/OOI/97 & UAB/004/97	Molecular typing of the West Lothian sub-clone of <i>E. coli</i> O157:H7	04/01/97	09/30/97	SERAD

UAB/005/97	Improved techniques for the rapid detection of <i>E. coli</i> O157 in foods	04/01/97	03/29/00	SERAD
UAB/007/99	Survival and dispersal of <i>E. coli</i> O157 in Scottish agricultural soils, and potential for contamination of private water supplies	04/01/99	03/31/02	SERAD
UAB/006/97	Characterisation of commensal and pathogenic <i>E. coli</i> populations in cattle	04/01/97	03/29/00	SERAD
UMA/001/95	Rapid luminescent detection and enumeration of low numbers of viable specific microorganisms	01/01/96	12/31/98	SERAD
URG/001/96	Generic, non-culture method for the detection of viable microorganisms	04/01/96	03/31/99	SERAD
K/MRS/50/C2601	Epidemiology and laboratory studies of <i>E. coli</i> O157	06/01/97	05/31/99	SODoH
K/MRS/50/C2624	Gut mucosal immunity and <i>E. coli</i> O157	01/01/98	12/31/99	SODoH

Total Number of projects = 70

A 1.3 Research and Development projects which started after the completion of the discussion paper.

Project Code	Project Title	Start Date	End Date	Funder
BFP11341	The Molecular Basis of Acid Habituation in Commensal and Pathogenic <i>E. coli</i>	15/08/99	14/08/02	BBSRC /SERA D
FS3503	Pathogens in organic wastes: their levels and survival both during storage & following application to agricultural land	01/07/99	31/12/02	MAFF
FS3506	The levels of pathogens in abattoir wastes	01/11/99	30/09/01	MAFF
OZ0705	A Proteomic Approach to Identify Virulence Determinants of EHEC O157	01/08/99	31/07/02	MAFF
OZ0708	A Systems Analysis Methodology to Elucidate and Evaluate the Critical Control Points for <i>E. coli</i> O157:H7 in Cattle and Sheep from Farm to Abattoir	01/01/00	30/06/02	MAFF
VF0101	The use of Signature Tagged Mutagenesis and <i>in vivo</i> Expression Technology to study the Colonisation of Cattle by <i>E. coli</i> O157:H7	01/10/99	30/09/04	MAFF
VF0201	Veterinary Research Fellowship in Epidemiology	01/09/99	31/08/04	MAFF
VF0304	The Biology of Verotoxigenic <i>E. coli</i> : Interaction with Ruminants	01/10/99	30/09/04	MAFF

Total Number of projects = 8

Last updated: 1 March, 2000

A 1.4 Food Surveillance Projects.

Project Title	Year	Funder
Microbiological analyses of beef cattle in six of the nine EU-approved abattoirs in Northern Ireland - Phase II	1997-1998	DANI
Surveillance of the microbiological status of raw cow's milk on retail sale	1995-1996	DH
<i>E. coli</i> O157 Survey - Part A - Cattle faeces	1994-1995	DHSS/DANI
<i>E. coli</i> O157 in cattle in Northern Ireland (carcass samples)	1997	DHSS/DANI
Microbiological examination of butchery products and butcher premises in the UK	1997	LACOTS/PHLS
Cold ready-to-eat meats from catering premises	1998	LACOTS/PHLS
RTE National Study: Part 2	1996	MAFF
RTE National Study: Part 3	1996	MAFF
RTE National Study: Part 4	1996	MAFF
Study of <i>E. coli</i> in unpasteurised milk cheeses on retail sale	1997	MAFF
Study of <i>E. coli</i> in unpasteurised cows' cream	1997	MAFF
Study of <i>E. coli</i> in beef, lamb and pork minced meat on retail sale	1997	MAFF
Study of unpasteurised sheep and goats' drinking milk	1997-1998	MAFF
Study of unpasteurised sheep, goats' and buffaloes' milk	1998	MAFF
Local survey of <i>E. coli</i> O157 in butchery products	1997	NIPHL
The microbiological quality of raw sausages sold in the UK	1994-1995	PHLS
EC Co-ordinated Food Control Programme Study of the microbiological quality of ready-to-eat dried and fermented meat and meat products	1996	PHLS
The public health implications of retail green top (unpasteurised) cows' milk sold at retail	1996-1997	PHLS
Health risks associated with raw goats' and ewes' milk on retail sale	1998	PHLS
The microbiological quality of imported unprepared whole lettuce	1998	PHLS
Microbiological examination of halal butchery products and butcher premises	1998	PHLS
<i>E. coli</i> O157 in meat/meat products, raw milk, unpasteurised cheeses	1997	SO
Bacteriological survey of food surfaces and meat slicers in retail premises	1997	SO

Total Number of projects = 23

Last updated: 1 March, 2000

A 1.5 Organisations which constitute the membership of the Microbiological Safety of Food Funders Group (MSFFG)

BBSRC	Biotechnology & Biological Science Research Council
DANI	Department of Agriculture for Northern Ireland
DH	Department of Health
DHSS, NI	Department of Health and Social Services, Northern Ireland
MAFF	Ministry of Agriculture, Fisheries and Food
SERAD	Scottish Executive Rural Affairs Department
SODoH	Scottish Office Department of Health