

ADVISORY COMMITTEE ON THE MICROBIOLOGICAL SAFETY OF FOOD

DISCUSSION PAPER

UK RESEARCH RELATING TO VEROTOXIGENIC *E. COLI* SUPERSHEDDING

The attached paper is a summary of recently completed and on-going research projects funded in the UK with relevance to the supershedding of *E.coli* by cattle and other ruminants. This summary focuses on research programmes funded by the FSA and Defra and supplements a presentation to be made to the Committee on current knowledge on supershedding and its role in transmission of human Verotoxigenic *E.coli* (VTEC) infection by Dr Chris Low of the Scottish Agricultural College (SAC). **Please note that Annex 1 of the attached paper¹ is a published paper and as such may be referenced but not published on websites. It is for Members Use Only and should not be photocopied.**

At the 24 September ACMSF meeting, the Committee considered the findings of the Public Inquiry into the *E.coli* O157 outbreak in South Wales in 2005, and subsequent work being taken forward through the FSA's Food Hygiene Delivery (FHD) Programme. This work includes a project to explore ways of reducing contamination of food animals by *E.coli* O157 on farm, in line with Recommendation 24 of the Enquiry to explore the feasibility of identifying "supershedder" cattle on farms as a potential means of reducing the likelihood of spreading *E.coli* O157 to other cattle.

Members are invited to note the current state of knowledge and on-going research in this area, and advise on priorities for future work to address this recommendation.

Secretariat
December 2009

¹ Margo Chase-Topping, David Gally, Chris Low, Louise Matthews and Mark Woolhouse. Super-Shedding and the link between human infection and livestock carriage of *Escherichia coli* O157. Nature Reviews **Vol 6** 904-912 (2008)

ADVISORY COMMITTEE ON THE MICROBIOLOGICAL SAFETY OF FOOD
RESEARCH RELATING TO VEROTOXIGENIC *E.COLI* SUPERSHEDDING

The Issue

1. To brief the Committee on current knowledge on supershedding and its role in transmission of human Verotoxigenic *E.coli* (VTEC) infection.
2. To update the Committee on recently completed and on-going research projects with relevance to the supershedding of VTEC by cattle and other ruminants, focussing on those funded by the FSA and Defra.
3. To ask the Committee to advise on priorities for future work which would address the recommendation on supershedding made in the Public Inquiry into the *E.coli* O157 outbreak in South Wales in 2005.

Background

4. At the 24 September ACMSF meeting, the Committee was briefed on the findings of the Public Inquiry into the *E.coli* O157 outbreak in South Wales in 2005, and the FSA's response to the recommendations made in the Report of the Inquiry.
5. Through its Food Hygiene Delivery (FHD) Programme, the FSA aims to take the lead in addressing all of the Report's findings and recommendations that lie within the Agency's remit. In response to Recommendation 24 of the Report (to explore the feasibility of identifying "supershedder" cattle on farms as a potential means of reducing the likelihood of spreading *E.coli* O157 to other cattle), the FSA has developed a workstream to explore ways of reducing contamination of food animals by *E.coli* O157 on farm.
6. At the 24 September meeting, the FSA asked the Committee to advise on what future research, interventions and education strategies should be taken forward in order to address this recommendation. As a starting point, it was suggested that a review was undertaken of relevant VTEC research to date. Annex 1 provides the most recent review on this subject which was published in Nature Reviews in December 2008.

UK Research on Supershedders

7. Over the past 10 years, a significant programme of research has been funded in the UK in the area of VTEC shedding by livestock and its impact on human infection. A list of key past and current UK research projects with relevance to supershedding is provided in Annex 2. A presentation summarising current knowledge in this area (to be given by Dr Chris Low of the Scottish Agricultural College), is provided in Annex 3.

History

8. Much of the UK research in this area has been based in Scotland and started with work funded by the then Scottish Executive following Professor Hugh Pennington's report on the circumstances leading to the *E.coli* O157 outbreak in 1996 in Wishaw, Central Scotland, which recommended research '*into the incidence/prevalence of E.coli O157 in Scottish cattle and other animals and the biology of its carriage*'.
9. Professor Pennington's report was also followed in 1999 by a £3.5 million research programme funded by the Wellcome Trust International Partnership Research Award in Veterinary Epidemiology (IPRAVE), titled 'Epidemiology and evolution of enterobacteriaceae infections in humans and domestic animals'. This project was a collaborative multi-disciplinary venture involving over 30 researchers from institutions in Scotland, elsewhere in Europe and North America. This 5 year programme included a cross-sectional study of 450 farms in Scotland and resulted in the first publications that described supershedding.
10. The IPRAVE programme coincided with a Defra Veterinary Pathology Fellowship (1999-2004) awarded to the University of Edinburgh, which identified the site of *E.coli* O157 colonisation in cattle. Exchange of information between these two key research projects led to the conclusion that supershedding is dependent on colonisation at the terminal rectum.
11. In addition, FSA Scotland funded a programme of work between 2002 and 2007 to complement the IPRAVE studies on cattle. This included projects to examine the seasonality of *E.coli* O157 shedding and prevalence of non-O157 VTEC in cattle. Further studies were also commissioned by FSA Scotland to determine the prevalence and concentration of *E.coli* O157 and non-O157 VTEC in sheep.
12. Defra have funded two projects under the Veterinary Training and Research Initiative (VTRI) which investigate general aspects of *E. coli* O157: VT0102 includes research into bacterial regulation and mechanisms which allow colonisation and adhesion of *E. coli* O157 at the terminal rectum in cattle; VT0103 investigated the general ecology of VTEC and the role of VTEC O157 in disease in terms of diversity. Both projects are due to conclude in 2009.

On-going and recently commissioned research

13. Two Defra funded research projects are currently underway which aim to develop control measures targeted at supershedding animals. One of these has identified a successful antigen combination for the development of a vaccine that can protect cattle against *E.coli* O157 colonisation and reduce shedding levels. Another project is conducting field trials to evaluate a cost effective treatment intervention for reducing shedding in finishing beef cattle. These projects are due to be completed in 2010.
14. An extensive, multidisciplinary research project, funded by the Rural and Economic Land Use (RELU) programme, is currently being funded to investigate mitigation strategies to reduce the risk of *E.coli* O157 in rural communities. This project includes microbiological and social science elements, and includes a workpackage which will use models to simulate the impact of interventions which target high shedding animals on levels of human infection through food and environmental exposure routes. Further work packages aim to establish the impact of the survivability of *E.coli* O157 in the farm environment to infectivity in cattle, and how the risks of VTEC infection are managed by rural stakeholders.
15. FSA Scotland has recently agreed to co-fund a PhD studentship with the University of Aberdeen, National Farmers Union Scotland (NFUS) and Quality Meat Scotland (QMS), which aims to investigate the findings of previous studies conducted in Scotland which indicated that the feeding of sheep with root crops led to a decrease in shedding of *E.coli* O157. This has the potential to lead to a practical mitigation step that can be applied at source (by the farmer) for the reduction of *E. coli* O157 numbers in animals destined for the foodchain and in the farm environment.

ACMSF Action

16. The Committee is invited to consider the current state of knowledge on supershedding and its role in the transmission of VTEC through the food chain, and to advise on future research, interventions and education strategies that should be taken forward to address Recommendation 24 of the Inquiry Report into the *E.coli* O157 outbreak in South Wales in 2005.

Secretariat
December 2009

Past and Current UK research projects with relevance to Supershedders

1. Welcome Trust International Partnership Research Award in Veterinary Epidemiology (IPRAVE)

Title: Epidemiology and evolution of enterobacteriaceae infections in humans and domestic animals
Duration: 1999-2003
Principal Investigator: University of Edinburgh

The aims of this programme were to understand the epidemiological relationships between enterobacteria in different hosts and host populations, to examine spatiotemporal distributions, molecular epidemiologies, transmission routes and risk factors, and to investigate the persistence of enterobacteria populations at different spatial scales. It also studied the distribution and movement of antibiotic resistance and virulence factors between different hosts and host populations, and explored the likely impact of changes in the management of enterobacteria infections through the development of statistical and mathematical models of the dynamics of these infections.

Individual project components included:

- Transmission dynamics in the field: epidemiological studies on Scottish beef cattle farms.
- Peri and post harvest risk identification and quantification of foodborne transmission : forward and backward tracing from farm to human outbreaks.
- Transfer of antibiotic resistance and virulence genes between host populations: molecular genetics studies of field material.
- Novel molecular approaches to study enterohaemorrhagic *E. coli* (EHEC): molecular biology of *E. coli* O157 and other VTEC.
- Population dynamics of antibiotic resistance and virulence plasmids and shigatoxin-encoding phages : experimental studies of bacterial population dynamics.
- Epidemiological studies of the distribution and spread of antibiotic resistance on Scottish cattle farms.
- Maintenance of central library of biological material and central database.

2. FSA Scotland

Title: SO1018 Quantifying the seasonality of *E. coli* O157 shedding (concentration and prevalence) in cattle and estimating its effect on the number of cases of food poisoning.
Duration: January-May 2003
Contractors: University of Aberdeen

The majority of *E. coli* O157 infections in humans occur during the summer months and the fewest during the winter. A study conducted in Scotland during summer 2002 showed that 7% of the cattle tested were positive for *E. coli* O157 in their faeces. Project SO1018 was commissioned to repeat the study during January to March 2003 to allow a comparison to be made between the concentration and prevalence of *E. coli* O157 shed by cattle during the summer and winter.

The study indicated that prevalence of *E. coli* O157 in cattle was greater during the cooler months compared to the warmer months, which does not agree with the seasonality of human infections. High shedding animals were found in similar numbers (approximately 1 in 200) during both the winter and summer months. However, the high shedding animals in the summer appeared to shed a significantly higher number of *E. coli* O157 compared to those identified during winter.

Molecular typing indicated that >90% of the *E. coli* O157 isolated could potentially cause disease in humans and also that the genetic profile of the organisms found in the cattle were similar to those found in humans. These findings suggested that cattle were a potential source of *E. coli* O157 infection in humans.

It was postulated that the higher number of *E. coli* O157 shed during the warmer months by the high shedding cattle could partly explain the higher number of clinical cases identified at this time of year. It was concluded that a method for removing these high shedding animals from the food chain or causing them to shed less *E. coli* O157 could lead to a reduction in the risk of food poisoning.

Title: SO1014 Prevalence of faecal shedding on Scottish beef cattle farms of verocytotoxigenic *Escherichia coli* serotypes: O26, O103, O111 and O145.
Duration: January 2002-June 2004
Contractors: Scottish Agricultural College

In Continental Europe, *E. coli* isolates of serogroups other than O157 also make a significant contribution to human diarrhoeal disease. This project studied the prevalence of non-O157 VTEC *E. coli*, in the faeces of Scottish beef cattle. The project built on an existing project funded by the Wellcome Trust through their IPRAVE (International Partnership Research Award in Veterinary Epidemiology) study, and aimed to lead to a better understanding of the relative potential risk of these organisms entering the Scottish Food Chain.

The findings provided the proportion of Scottish finishing cattle shedding *E. coli* of types O26, O103, O111 and O145. The results indicated that VTEC strains of types O103, O111 or O145 were uncommon or absent in cattle sampled in Scotland. In contrast, the type O26 VTEC were considerably more common and at farm level the 10% prevalence was close to the 14.7% found for *E. coli* O157. Additionally, there were clear seasonal patterns with the lowest number of non-O157 VTEC positives found in the spring and the highest during summer and autumn. This was opposite to previous results for *E. coli* O157 where more positives were identified when animals were kept inside buildings in the cooler months rather than out to pasture during the summer months.

Title: S14005 Prevalence and Concentration of *Escherichia coli* O157 and other VTEC in sheep presented for slaughter in Scotland.
Duration: July 2005 to September 2007
Contractors: Scottish Agricultural College

Since the late 1980s, rates of infection of *E. coli* O157 in certain regions of Scotland have been substantially and constantly higher than those seen in England & Wales. In Continental Europe, *E. coli* isolates of serogroups other than O157 also make a significant contribution to human diarrhoeal disease. Domestic ruminants, especially cattle and sheep, are major reservoirs of VTEC. Most epidemiological work on VTEC has focused on cattle despite sheep being identified on a number of occasions as the source of outbreaks of human VTEC infection in Scotland. This study was undertaken to determine the prevalence of *E. coli* O157 and the major non-O157 VTEC in sheep at slaughter, and to ascertain whether there were seasonal or regional differences. The project sampled 1,082 sheep at slaughter at four different slaughterhouses in Scotland from July 2005 to June 2006.

This research found that the age of the animal and season have a greater impact on prevalence of *E. coli* O157 carriage than the regional origin of the flock with the highest risk observed during July to September in sheep that were adults or one year old. There were 33 animals positive for *E. coli* O157 and in seven of these animals the actual numbers of O157 was more than 1000 per gram of dung. This was similar to results from cattle where animals, termed supershedders, producing these high numbers of *E. coli* O157 in the dung are believed to be important for the spread of the bacterium in groups of animals. VTEC strains of serogroups O103, O111 or O145 were found to be of low prevalence or absent from sheep at slaughter. The prevalence of *E. coli* O26 carriage in this study was similar to that of *E. coli* O157 however only 17% of *E. coli* O26 isolates possessed the verocytotoxin genes, whilst virulence genes were identified in 81% of the O157 sheep isolates. These findings indicated that in Scotland, sheep appear to be less likely than cattle to be a source of the VTEC O26 strains in humans.

Title: PG1007 Pathogenic Potential of *Escherichia coli* O26 and Sorbitol-Fermenting *Escherichia coli* O157:NM (FSA post-graduate scholarship scheme-awarded).

Duration: 2004-2007

Contractors: University of Edinburgh

The study initially characterised *E. coli* serogroup O26 isolates recovered from human infections in Europe and Scotland and isolates collected from Scottish cattle with the objective of identifying factors which may allow strains to cause more serious clinical disease. On the basis of phenotypic results it appeared that the majority of Scottish cattle *E. coli* O26 isolates have different characteristics to those causing human disease, and this could explain the low incidence of *E. coli* O26 human infections in Scotland. However, a Scottish cattle isolate was identified which, for the genotypic characteristics investigated, was indistinguishable from the isolate recovered from a patient with HUS. The findings suggested that the differences in pathogenic potential between isolates were likely to be due to horizontally acquired DNA, including *vtx*₂ carriage and the O-island-phage-associated effector protein repertoire.

During the research an outbreak of human infection with atypical sorbitol-fermenting (SF) *E. coli* O157 occurred in Scotland and the studies were expanded to compare this emergent pathogen to non-sorbitol-fermenting (NSF) *E. coli* O157 strains. While no evidence of toxin or toxin expression differences between the two VTEC O157 groups was found, the SF VTEC O157 strains adhered at significantly higher levels to a human colonic cell line. Under the conditions tested, curli fimbriae were shown to be the main factor responsible for the increased adherence to Caco-2 cells. It was concluded that the capacity of SF VTEC O157 strains to express curli at 37°C may have relevance to the

epidemiology of human infections as curliated strains could promote higher levels of colonization and inflammation in the human intestine. In turn this could lead to increased toxin exposure and an increased likelihood of progression to HUS.

University of Aberdeen PhD studentship (co-funded by FSAS, NFUS and Quality Meat Scotland)

Title: Effect of feed on *E. coli* O157 shedding in ruminants and source attribution of human infection using MLVA typing.
Duration: October 2009-September 2012
Contractors: University of Aberdeen

Previous studies have reported that diet can influence the concentration of *E. coli* O157 shed by sheep. Pilot work conducted in Grampian has demonstrated that whilst sheep fed on root crops/silage/barley in the winter/early spring had a similar flock prevalence of *E. coli* O157 to animals previously sampled at pasture during the summer months, they were not found to shed target *E. coli* O157 at such high concentrations (highest concentrations detected in winter were $10^3/g$ compared to $10^6/g$ in summer). Furthermore, the majority of *E. coli* O157 isolates shed in winter were found to be atoxigenic (lacking *vt1* and *vt2* toxin genes) as tested by PCR and confirmed by Vero cell assay.

Feeding ruminants with root crops could be a practical mitigation step in reducing shedding of *E. coli* O157 as root crops (e.g. turnips) are routinely used as ruminant feed. In North East Scotland, turnips are also fed to cattle but the effect of this feeding practice on their bacterial shedding has not yet been studied. At this stage it is unknown whether winter feed was contributory to the levels and toxigenic profiles of *E. coli* O157 and if so, which component of the feed might be effective.

This studentship will conduct more intensive farm based testing during the autumn and winter months to validate the previous findings. The study will also investigate the factors in the feed that impact on *E. coli* O157 numbers shed by farm animals and will provide comprehensive data on shedding patterns and MLVA profiles of *E. coli* O157 to identify any relevant links to human infection.

2. Defra

Title: OZ0712 *E. coli* O157 interventions and control.
Duration: 2003-2007
Contractors: Scottish Agricultural College (SAC)

This study examined treatment options to reduce or treat colonisation of cattle. It established that *E. coli* O157:H7 is not a strict commensal of the bovine host but colonises and induces pathological changes and stimulates a transient immune response. It also confirmed that the principle site of colonisation in cattle is the terminal rectal mucosa, and demonstrated that the bacterial type III secretion system is essential for this colonisation. This project showed that transmission between calves could be demonstrated in an experimental model.

Faecal levels of this bacterium were found to be reduced or completely eliminated by direct application of chlorhexidine to the rectal mucosa. A dip stick test was also used to allow rapid identification of individuals with high level faecal carriage of the organism.

This work has substantially increased our knowledge of *E. coli* O157:H7 colonisation of cattle. The finding of terminal rectal colonisation has been confirmed and is now internationally accepted. This has led to considerable advances in our understanding of the mechanisms of host colonisation and allowed us to examine how cattle respond to infection. Knowledge of colonisation and shedding patterns has been shared with mathematical modellers. The findings (Matthews et al., 2006) show that identifying and eliminating terminal rectal colonisation, or preventing terminal rectal colonisation would be effective means to disrupt spread of the organism in cattle. This project provided a means to identify animals with high level carriage of *E. coli* O157:H7 and also to successfully treat them.

Title: LK0666 Vaccination strategies for control of enterohaemorrhagic *Escherichia coli* O157:H7 in cattle.
Duration: 2005-2008
Contractor: University of Edinburgh

This project identified a successful antigen combination that can protect cattle against *E.coli* O157 colonisation and reduce shedding levels. A patent is pending on the use of this antigen combination in vaccine development. Proposals for further work (commercially confidential) are currently being considered to develop this further and to conduct trials, along with other vaccine approaches in collaboration with the LINK industrial partners and other companies currently involved in the development of vaccines targeted at *E.coli* O157 in cattle. Whilst developments in this area have been promising, it has been recognised that cost of uptake and drivers for use may present a challenge for vaccine implementation, since *E.coli* O157 does not cause disease in the animal.

Title: OZ0714 Development a cost effective and practical method to reduce *E.coli* O157 infection in cattle prior to slaughter.
Duration: 2008-2010
Contractor: Scottish Agricultural College (SAC)

This study intends to develop a longitudinal sampling of farms and to examine the relationship of persistence to faecal shedding.

It is known that a small percentage of cattle intermittently shed high levels of the bacteria in the faeces when an area in the rectum is colonised by *E. coli* O157, though these animals will show no signs of disease. Experiments have shown that the bacteria can be removed by washing out the rectum with a chlorhexidine solution. This study aims to investigate the effectiveness of this treatment intervention in groups of finishing beef cattle. A pilot study was carried out on two farms in the winter of 2008-09 with promising results and further beef finishing units have now been recruited to take part in a further field study during the winter of 2009.

3. Rural Economy and Land Use Programme (RELU)

Title: Reducing *E.coli* O157 Risk in Rural Communities
Duration: 2007-2010
Principal Investigator: University of Aberdeen

This project brings together the disciplines of geography, sociology, economics, medicine, microbiology, ecology and food science to carry out the research needed to

advise a range of stakeholders with the scientific information needed to reduce the risk of human *E.coli* O157 infection in UK rural communities.

Current outbreaks of human diseases and food safety concerns caused by food borne pathogens such as *E. coli* O157 flag a serious lack of knowledge and understanding about the factors which determine the numbers and spread of these human food-borne pathogens in rural and agricultural environments. The pathogens mainly enter the food chain from faecal contamination of meat products, but there are an increasing number of outbreaks linked to direct contact with contaminated water, soil and livestock. The research aims to provide information to ensure rural policies minimise the risks to rural communities from *E. coli* O157. Through 6 integrated work packages, a number of key questions will be addressed. These include science led questions (e.g. why do farm workers appear to less likely to be infected than people living in towns? Is it linked to long-term, but low level exposure? It is known that *E. coli* O157 survives for long periods in the farm environment, but after this time is it still able to cause disease to humans? Which farm factors pose the greatest risk to human and animal (re)infection?), as well as social science led questions (e.g. how is *E. coli* O157 risk seen, communicated and managed by stakeholder groups? How much does *E. coli* O157 human infection cost the UK and what would any socially acceptable measures to minimise risk cost?),

Two case study, rural areas with contrasting incidence of *E. coli* O157 have been chosen - one area has a high incidence of human O157 infection (The Grampian Region of North East Scotland) and the second has a relatively low incidence (North Wales). Both have been previously studied by the research team and a number of strong networks exist for surveying and studying key groups such as farmers, contractors, slaughterhouses, butchers, caterers, and other rural stakeholder groups.

The 6 work packages correspond with the 6 major objectives of the study:

1. Understand awareness, attitudes and behaviour of rural stakeholders to *E. coli* O157 risk.
2. Study links between survival of this pathogen and its carriage, often without disease, within rural communities.
3. Investigate if the pathogen can still be infective to humans (and cattle) after long term survival in the environment.
4. Identify the costs of ways to reduce infection levels of *E. coli* O157 in rural areas.
5. Determine if the economic and social costs are acceptable to stakeholders.
6. Produce detailed risk management strategies on rural *E. coli* O157 infection for government policy.

As is all too apparent from the most recent outbreaks in both Scotland and Wales that *E. coli* O157 disease can have a devastating impact on the lives of individuals (often children) and their families in rural communities, it is hoped that this work will lead to policy ready measures to reduce risk in rural communities. Although the research focuses on *E. coli* O157, the findings and conclusions will also be generally applicable to minimising the risk associated with many other disease causing human pathogens in the UK rural environment.