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ACM/1341

ADVISORY COMMITTEE ON THE MICROBIOLOGICAL SAFETY OF FOOD

STEC Research in Scotland

Introduction

1. Since Food Standards Scotland (FSS) was established in April 2015, understanding the transmission of Shiga toxin-producing *E. coli* (STEC) has been one of its research priorities. FSS's research programme on STEC has developed from an earlier programme commissioned by the Food Standards Agency (FSA) and FSA in Scotland to address recommendations made in the inquiry of the *E. coli* O157 outbreak in South Wales in 2005[1]. It also supports the wider objectives of Scottish Government's STEC Action Plan, a multi-disciplinary initiative aimed at reducing the burden of STEC infection in Scotland[2].

2. To date, the results of FSS's research programme on STEC have been used to inform policy in the following areas:
   - To support risk management actions following the detection of STEC in food;
   - To develop industry and local authority guidance on managing the risks of STEC in food production;
   - To improve our understanding of STEC transmission via different host species and manufacturing processes;
   - To generate evidence to support risk assessment;
   - To inform appropriate interventions for reducing STEC in food, including the efficacy and feasibility of a vaccine against *E. coli* O157 in cattle.

3. This paper updates the Committee on FSS's STEC research programme, which is designed around three themes: Understanding the source, Understanding STEC risks in the food chain, and Understanding the epidemiology of STEC in Scotland. We have outlined the findings of seven projects commissioned by FSS and its partners between 2015-2020 and some of the key findings and outputs generated by this work to date.

Background - STEC Infection in Scotland

4. Clinical STEC infection has been consistently higher in Scotland compared to the other UK countries for a number of years. Public Health Scotland[3] undertakes enhanced surveillance for STEC in collaboration with the Scottish *E. coli* O157/STEC Reference Laboratory (SERL) and NHS board health protection teams. In 2019, there were 150 cases of *E. coli* O157, and 108 of non-O157

[1] http://www.reading.ac.uk/foodlaw/pdf/uk-09005-ecoli-report-summary.pdf
[2] https://www.gov.scot/publications/vtec-e-coli-o157-action-plan-scotland-2013-2017/
[3] https://www.hps.scot.nhs.uk/web-resources-container/stec-in-scotland-2019-enhanced-surveillance-and-reference-laboratory-data/

reported to HPS, representing a slight decrease from 2018. Case numbers were highest during the summer months and higher rates are observed in children under 5 – consistent with trends seen in previous years.

5. Since the formation of FSS there have been two notable outbreaks of STEC O157 in Scotland. One occurred in 2015 and was linked to the consumption of venison products in the home[4], and a second occurred in 2016 and was associated with the consumption of an unpasteurised cheese[5]. During the investigation of both of these outbreaks it was noted that there were important evidence gaps relating to the control of STEC in these products.

FOOD STANDARDS SCOTLAND'S RESEARCH PROGRAMME ON STEC

Theme 1: Understanding the source

Background and previous research

6. Much of the UK research in this area has been based in Scotland and started in the 1990s with work funded by the then Scottish Executive following Professor Hugh Pennington's report[6] on the circumstances leading to the *E. coli* O157 outbreak in 1996 in Wishaw, Central Scotland. This recommended research into *'the incidence/prevalence of E. coli O157 in Scottish cattle and other animals and the biology of its carriage*'.

7. Professor Pennington's report was followed in 1999 by a £3.5 million research programme funded by the Wellcome Trust International Partnership Research Award in Veterinary Epidemiology (IPRAVE), entitled '**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 five year programme included a cross-sectional study of 450 farms in Scotland and resulted in the first publications that described '*super-shedding*' - a phenomenon whereby *E. coli* O157 was excreted from cattle at particularly high levels (approximately 10^3-10^4 cfu/g).

8. 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 super-shedding is dependent on colonisation at the terminal rectum.

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[4] https://www.hps.scot.nhs.uk/web-resources-container/national-outbreak-of-escherichia-coli-o157-phage-type-32-in-scotland-september-october-2015-report-of-the-incident-management-team/

[5] https://www.hps.scot.nhs.uk/web-resources-container/national-outbreak-of-escherichia-coli-o157-phage-type-2128-in-scotland-july-september-2016/

[6] The Pennington Group: Report on the circumstances leading to the 1996 outbreak of infection with *E. coli* O157 in Central Scotland, the implications for food safety and the lessons learned. Edinburgh: The Stationery Office, 1997

9. In addition, the FSA in 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[7,8,9]. Further studies were also commissioned by the FSA in Scotland to determine the prevalence and concentration of *E.coli* O157 and non-O157 VTEC in sheep[10].

10. Research in this area was re-instated following the public inquiry into the *E. coli* outbreak in Wales in 2005[11]. One of the recommendations made in the inquiry report was:

    The feasibility of identifying "super-shedder" cattle on farms should be explored as a potential means of reducing the likelihood of spreading E. coli O157 to other cattle.

    Evidence generated through the earlier IPRAVE work had also suggested an association between serious human illness and super-shedding. It was therefore identified that further research was needed to understand the role of super-shedding and how to mitigate this phenomenon as a means of controlling environmental and foodborne transmission.

Current research focus

11. The project ***E. coli* O157 Super-Shedding in Cattle and Mitigation of Human Risk**[12] began in 2013 and the final report was published in 2018. The study had four main objectives, (i) To investigate the prevalence of *E. coli* O157 in cattle in farm surveys of Scotland and England & Wales. (ii) To sequence cattle and human *E. coli* O157 isolates, determining their population structures and relationships with super-shedding and human disease. (iii) To determine the excretion dynamics and transmission frequencies between cattle of wild type *E. coli* O157 strains under controlled experimental conditions. (iv) To test an intervention using a super-shedding strain and model the impact of an intervention based on data generated in this programme.

12. The study found that over the last decade levels of *E. coli* O157 in cattle have remained relatively constant in Scotland and are equivalent to those in England & Wales. Based on faecal pat sampling, approximately 20% of farms and 10% of animals were positive for *E. coli* O157. The diversity of O157 subtypes in cattle was much greater in England & Wales compared to Scotland, which had a higher level of a certain subtype (PT21/28) producing the Stx2a toxin. This particular type of *E. coli* is associated with super-shedding and severe human

[7] https://www.foodstandards.gov.scot/publications-and-research/publications/quantifying-the-seasonality-of-e-coli-o157-shedding-concentration-and-prev
[8] https://www.foodstandards.gov.scot/publications-and-research/publications/comparison-of-human-and-cattle-e-coli-o26-isolates-by-pulsed-field-gel-ele
[9] https://www.foodstandards.gov.scot/publications-and-research/publications/prevalence-of-faecal-shedding-on-scottish-beef-cattle-farms-of-verocytotoxi
[10] https://www.foodstandards.gov.scot/downloads/Report_to_FSA_Scotland_On_Project_S14005.pdf
[11] http://gov.wales/docs/dhss/publications/150618ecolireporten.pdf
[12] https://www.foodstandards.gov.scot/downloads/Super-shedders_-_FINAL_version_for_publication.pdf

infection. Exposure to PT21/28 may therefore help to explain the higher human incidence of O157 infection in Scotland compared to that in England & Wales.

13. For the *E. coli* O157 subtype PT21/28, it was demonstrated that the majority of human isolates can be traced back genetically to recent ancestors present in the British cattle population.

14. It was demonstrated under experimental conditions that a PT21/28 *E. coli* O157 isolate was excreted from cattle at higher levels than an alternative subtype, PT32. It was also shown that the presence of Stx2a plays an important role in establishing high level excretion in nearby exposed animals - meaning this toxin is important for maintaining specific *E. coli* O157 strains in the cattle reservoir through enhanced transmission. The mechanism behind the advantage conferred by the Stx2a toxin in the ruminant reservoir remains to be determined.

15. The final part of the study showed that a vaccine developed by the team significantly reduced excretion levels of a *E. coli* O157 *stx*2a+ isolate from cattle and limit its transmission to other in-contact calves. The vaccine development is currently being taken forward by Roslin Technologies Ltd[13] and is now at the stage of field trials.

Theme 2: Understanding STEC risks in the food chain

Internalisation of STEC into plant tissue

16. Over the past 10 years, there have been a number of UK outbreaks of STEC which have been linked to the handling or consumption of fresh produce, which have raised questions regarding the efficacy of washing as a control measure. It is has been known for some time that foodborne pathogens such as STEC and salmonella can exist on both the external and internal surfaces of plants, however there were gaps in our knowledge of how the internalisation of pathogens was related to the risk of transmission from these types of food vehicles. To examine this, FSS and Food Standards Agency (FSA) co-funded a study to investigate the internalisation of pathogens into plants - **The capacity and pathogenic potential of bacteria that internalise into plant tissue**[14].

17. The study concluded that current washing practices used in post-harvest production do not remove or inactivate any internalised bacteria and are ineffective at removing all external bacteria from plant surfaces. The study also revealed that internalised *E. coli* were not compromised in their ability to interact with and bind to human gut epithelial cells and hence are still likely to be able to cause disease if ingested. These conclusions reinforce the importance of Good Agricultural Practices, such as use of clean irrigation water, and have been used

[13] https://www.roslininnovationcentre.com/news/roslin-technologies-signs-collaboration-agreement-to-develop-e-coli-vaccine

[14] https://www.foodstandards.gov.scot/downloads/The_capacity_and_pathogenic_potential_of_bacteria_that_internalise_into_plant_tissue_-_March_2017.pdf

to strengthen guidance for producers such as in the online **Fresh Produce Tool**[15].

Controlling STEC in Raw Milk Cheeses

18. Following the outbreak of *E. coli* O157 in 2016 associated with a raw milk cheese, a number of recommendations were made by the multiagency Incident Management Team to improve the safety of such products and prevent any future outbreaks. This included the recommendation:

    During 2017 FSS and the Scottish Food Enforcement Liaison Committee (SFELC) to work with local authorities and the Specialist Cheesemakers Association to strengthen existing guidance and promote an understanding across the sector (particularly small producers) of potential risks associated with STEC and control measures and testing regimes required to manage the risk.

19. To inform the development of such guidance, FSS commissioned a literature **Review Of Controls For Pathogen Risks In Scottish Artisan Cheeses Made From Unpasteurised Milk**[16] to assess (i) classification of cheese types made in Scotland and key points for controlling bacterial contamination during the production of these products, (ii) the use of predictive modelling and challenge testing in validation, and (iii) the interpretation of microbiological test results & identification of trends.

20. The review provided a number of tools (such as HACCP flowcharts and microbiological criteria) which can be used by industry to inform the development or strengthen their Food Safety Management Systems. The evidence from the review was also used by FSS to assist the development of SFELC's **Guidance for Local Authority Enforcement Officers on the Production of Cheese from Unpasteurised Milk**[17] - for example to design a pragmatic approach to *E. coli* and STEC sampling for cheese and the raw milk used to make cheese.

STEC risks in wild venison production

21. A number of evidence gaps were also identified following an outbreak of *E. coli* O157 which occurred in Scotland in 2015, and was linked to venison products. Following this outbreak, FSS instigated a collaboration between industry, Scottish Government (the Rural and Environment Science and Analytical Services Division) and the research community to attempt to address these gaps with a view to enhancing food safety management in this sector. This resulted in

[15] http://freshproducetool.foodstandards.gov.scot/

[16] https://www.foodstandards.gov.scot/downloads/FSS_2017_015_-_Control_of_pathogens_in_unpasteurised_milk_cheese_-_Final_report_v_4.7_-_20th_November_2018_.pdf

[17] https://www.foodstandards.gov.scot/downloads/Guidance_for_Local_Authorities_%E2%80%93_Cheese_made_from_Unpasteurised_Milk_-_May_2019.pdf

the project **The risk of Shiga toxin-producing *E. coli* (STEC) contamination in wild venison**[18].

22. The work had three main objectives, (i) to map the venison industry in Scotland: (ii) a field survey to assess STEC prevalence in wild deer faeces in Scotland and sequence O157 isolates found, and (iii) a review of cross-contamination risks in the slaughter and processing stages of wild deer from the field to the larder. The project also supported an MSc student to sequence the non-O157 STECs isolated from deer faeces. The project is due to be published in late 2020.

23. The prevalence study was conducted on wild Scottish deer faeces between July 2017 and June 2018. Samples represented all four wild deer species in Scotland (Red, Roe, Sika and Fallow deer) from all regions of Scotland. Out of a total of 1,087 faecal samples analysed, three were positive for STEC O157 (0.34%). All three deer STEC O157 strains contained the *stx*2a and *eae* genes.

24. A risk factor analysis was undertaken using the published literature combined with a field and processing swabbing survey. Risk factors which predicted increased contamination of venison carcasses with *E. coli* and coliforms included visual contamination of the skin with faeces or dirt, wet and slimy carcasses, environmental temperatures >7°C and increased distance between cull location and Approved Game Handling Establishment.

25. The results of this study are currently being used to update best practice guidance for the wild venison sector. The focus of this guidance is the implementation of more robust hygiene precautions to prevent the faecal contamination of carcasses during processing, which is expected to minimise the risk of human STEC infections from wild venison.

The microbiological quality of minced beef on retail sale in Scotland

26. In 2018 FSS commissioned a **Survey of the microbiological quality of mince beef on retail sale in Scotland**[18]. The key aim of this work was to gain a better understanding of how STEC prevalence and shedding levels in cattle related to contamination risks in red meat processing. The data generated from this survey will be used to (i) assist in the development of industry and enforcement guidance, (ii) perform more accurate risk assessment of consumption of mince served less than fully cooked (such as rare burgers), and (iii) provide comparison between those types of STEC that have been sequenced previously in Scotland, such as those found on farm in the super-shedders work, those found in clinical illness and those found in other commodities, thus increasing our understanding of the STEC transmission cycle. The survey will also provide data on the relationships between generic *E. coli* and the presence of pathogens such as STEC and salmonella.

[18] https://www.foodstandards.gov.scot/publications-and-research/scientists-and-researchers/food-safety-research

27. Sampling was undertaken during the course of Jan – Dec 2019, targeting retailers across Scotland based on a statistical representation of market share. Analyses of these samples were undertaken to achieve the following aims: (i) to generate baseline data on the significant microbiological pathogens and hygiene indicator organisms present in beef mince on retail sale to the consumer in Scotland, (ii) to analyse any patterns of variation (e.g. seasonal or geographical) in order to identify any risk factors associated with microbial contamination, and (iii) to determine the presence of antimicrobial resistance (AMR) in the pathogens and a subset of 100 isolates of generic *E. coli* found.

28. The mince samples were screened for salmonella, campylobacter and STEC, as well as the hygiene indicator organisms *E. coli* and ACCs. The presence of AMR was determined using both phenotypic (disk diffusion and MIC) and genotypic methods (sequencing).

29. During the course of the survey, adverse results were reported back to retailers in real time to allow appropriate risk management to be undertaken. The results of the survey will be published in early 2021.

Theme 3: Understanding the epidemiology of STEC in Scotland

The diversity of clinical non-O157 STEC in Scotland

30. There is an increasing awareness in the scientific and medical communities of the importance of non-O157 STECs as the cause of significant mortality and morbidity in patients. Whilst much is now known about STEC O157, much less is known about O157, and yet in some European countries, a non-O157 is the most prevalent clinical STEC serotype. In Scotland, non-O157 STECs account for around 30% of all STEC infections.

31. To develop our understanding of the importance of non-O157 STEC, we commissioned the SERL to sequence its archive of 525 non-O157 STEC. The corresponding report **Whole Genome Sequence Typing And Analysis Of Non-O157 STEC**[19] details the complexity of non-O157 STEC infection in Scotland over the past 16 years.

32. *In silico* serotyping identified 88 different serotypes (where both O and H group were identified) among the *E. coli* isolates. The most common clinical non-O157s in Scotland are O26:H11 (27.0%) and O103:H2 (9.4%) – these are also two of the most common serotypes circulating worldwide. The most common *stx* gene subtype found was *stx1*a which found in 176 isolates (33.7%), and a total of 92 isolates (17.6%) carried AMR genes present in the Resfinder database

[19] https://www.foodstandards.gov.scot/downloads/WGS_Typing_and_Analysis_of_Non-O157_STEC_-_Jan_2020_v3.pdf

33. A molecular risk assessment was undertaken, in which the characteristics of each of the Scottish clinical isolates was assigned to a categorisation as proposed by the joint FAO/WHO expert group on STEC (JEMRA)[20]. Of the 517 isolates examined, 142 (27.5%) were classified as Level 1 or 2 (conferring the potential to cause HUS), 166 (32.2%) as Level 3 or 4 (potential to cause diarrhoea or bloody diarrhoea) and 209 (40.5%) as Level 5 (potential to cause diarrhoea).

34. In 2021, the findings from SERL's work will be incorporated into work being undertaken by PHS which will link the sequencing data from Scottish STEC strains to the clinical and epidemiological information collected from these patients through PHS's enhanced surveillance programme. This will include examining whether the clinical illness seen in Scottish patients infected with different STEC strains corresponded to the clinical outcome predicted by JEMRA. This will help advance our understanding of what defines a "pathogenic STEC" – a concept which does not yet have a full definition or scientific consensus.

35. Whilst the evidence continues to grow, the results of this study, i.e. the diversity of non-O157 STEC found in Scottish patients supports the basis for the FSS and FSA policy position on appropriate risk management action when STEC is detected in food i.e. that the presence of *any* STEC should be considered potentially pathogenic.

Estimating the burden of disease

36. In 2021 PHS will begin work to estimate the burden of clinical STEC and determine risk factors and clinical outcomes in the project **STEC: Estimating the burden of gastrointestinal infection in Scotland using data linkage**. This will build on a suite of linkage work undertaken by PHS to estimate the burden of disease for the main foodborne pathogens, including STEC, salmonella and campylobacter[21].

Knowledge gaps, issues and recommendations

37. Over the past 5 years FSS has filled a number of gaps in our knowledge of the transmission of STEC, and this knowledge has been used directly to support interventions for reducing risks to the food chain. FSS intends to continue to prioritise STEC in its future research programmes, working in collaboration with the FSA, Scottish Government, PHS and partners to address knowledge gaps in this area. With regard to addressing STEC at source, there is particular interest in the importance of sheep and dairy cattle as hosts in the transmission cycle. We have also recognised that it would be informative to adopt an "ecosystem

[20] Joint FAO/WHO expert meetings on microbiological risk assessment (JEMRA). Shiga toxin-producing *Escherichia coli* (STEC) and food: attribution, characterisation and monitoring (2018).
[21] https://www.foodstandards.gov.scot/publications-and-research/publications/campylobacter-epidemiological-data-linkage-study

approach” to the study of the transmission of STEC, for example by examining the relationships between different types of host and whether inter-species transmission occurs through processes such as co-grazing.

38. With regard to food safety management, there are still a number of technical difficulties for Food Business Operators associated with sampling and typing of STEC, particularly for non-O157 STEC, the testing for which is not easily available on a commercial basis. Our experience in dealing with outbreaks and incidents has indicated that further work is needed to improve the accessibility of testing for non-O157 in food, and to understand the value of testing for faecal indicator organisms (generic *E. coli* or O157) as proxy predictors of STEC contamination.

Action

39. ACMSF Members are invited to comment on research on STEC in Scotland at the October 2020 meeting, and propose any further evidence and information gaps for consideration for funding further research.

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