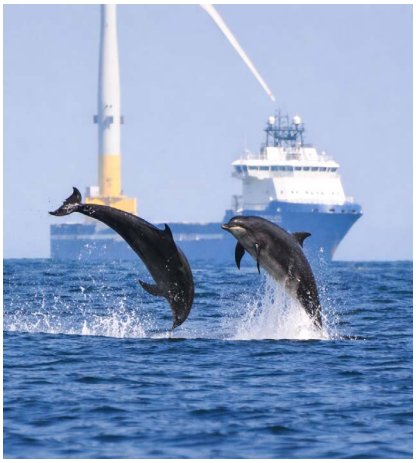


VTEC 2026

International Symposium On Shiga Toxin Producing *Escherichia coli* Infections Conference

SRUC

VTEC2026 ABERDEEN

1495 UNIVERSITY OF ABERDEEN

Contents

Welcome from the Chair

We are delighted to welcome the STEC, VTEC and E. coli community to Aberdeen for the 2026 VTEC conference. It has been three years since the last, excellent conference in Banff, Canada, and only the second time the meeting has visited Scotland. The Aberdeen meeting continues the spirit and ethos of the VTEC series, building on its established and prestigious heritage. STEC continues to be a priority pathogen across multiple geographies, impressing the need for a setting where all disciplines and expertise can gather together to discuss the key priorities and share the cutting edge knowledge. We are delighted that the full scope of One Health topics and disciplines is covered for STEC in Aberdeen and that the main symposium is supplemented by events with an applied focus. We hope you have a fulfilling experience, and make the most of your trip to historic Aberdeen and beautiful Scotland.

Professor Nicola Holden, SRUC.
Chair of the VTEC Local Organising Committee 2026

Local Organising Committee

Professor Nicola Holden - Professor of Food Safety, SRUC

Dr Lesley Allison - Principal Clinical Scientist Scottish E. coli Reference Lab (SERL), NHS Lothian

Dr James Connolly - MRC Research Fellow/Group Leader, Newcastle University

Dr Marianne James - Head of Risk Assessment, Food Standards Scotlands

Dr Sally Johnson - Consultant Paediatric Nephrologist, Newcastle Upon Tyne Hospitals NHS Foundation Trust

Dr Lesley Larkin - Consultant Healthcare Scientist, Public Health Scotland

Dr Janet Nale - Lecturer in Microbiology, SRUC

Professor Karen Scott - Principal Scientist Rowett Institute, University of Aberdeen

Dr Cristina Soare - Senior Lecturer, Edinburgh University

Prof Dr Nicole Van De Kar - Pediatric Nephrologist Radboudumc, Nijmegen, The Netherlands

International Steering Committee

The International Steering Committee are made up of members who have long-standing association with the VTEC conference series, most are previous organisers. Collectively, they cover all of the One Health areas for VTEC / STEC from human, animal and environmental health, and all disciplines from medicine, microbiology, epidemiology and policy.

Dr. Nicole van de Kar - Radboud University Medical Center, the Netherlands (chair)

Dr. Todd Riley Callaway - University of Georgia, USA

Dr. Gad Frankel - Imperial College London, United Kingdom

Dr. James Kaper - University of Maryland School of Medicine, USA

Dr. Stefano Morabito - Istituto Superiore di Sanità, Rome, Italy

Dr. Tim Mcallister - Agriculture and Agri-Food Canada, Canada

Dr. Herbert Schmidt - University of Hohenheim, Germany

Dr. Phillip Tarr - Washington University School of Medicine, USA

Dr. Haruo Watanabe - National Institute of Infectious Diseases, Japan

Dr. Iruka Okeke – University of Ibadan, Nigeria

Dr. Patricia M. Griffin - Centers for Disease Control and Prevention, USA (emeritus)

Dr. Mohamed A Karmali - Toronto, Canada (emeritus)

Dr. Flemming Scheutz - Denmark (emeritus)

Outline programme

Sunday 10/05/2026

15.00 – 17.00	**Registration**	
17.00 – 18.30	**Welcome and Opening Plenary Session** Opening keynote: Historical & Scottish perspective; patient-focused perspective; One Health links George Gunn (SRUC, chair); Sally Johnson (Newcastle NHS); Stefano Morabito (EURL-STEC)	P&J Live
18:30 – 20.30	Welcome Reception	P&J Live

Monday 11/05/2026

08.00 – 09.00	Registration	P&J Live
09.00 – 10.00	**Plenary Session One** Public Health: Epidemiology Sooria Balasegaram (UKHSA)	P&J Live
10.00 – 11.00	**Plenary Session Two** Pathogen Genomics and Evolution Lauren Cowley (University of Bath)	P&J Live
11.00 – 11.30	Refreshments	P&J Live
11.30 – 12.30	**Plenary Session Three** Public Health: Food Safety Alec Kyriakides (Independent Food Consultant)	P&J Live
12.30 – 13.30	Lunch	P&J Live
13.15 – 14.15	Pop Up Session 1: Post Infection Shedding Discussion	
13.30 – 14.30	**Poster Session 1 – Odd Numbers**	P&J Live
14.30 – 16.30	**Parallel Session A** Clinical Health: Patient treatment & early diagnosis Paula Coccia (Buenos Aries)	P&J Live

15.30 – 16.00	Refreshments	P&J Live
14.30 – 16.30	**Parallel Session B** Pathogen: Transmission (on-farm, wildlife, environment) Anne Allende (CEBAS-CSIC, Spanish National Research Council)	P&J Live
16.30 – 17.00	Flash **Poster Sessions A & B**	P&J Live
17.15	Buses from P&J Live to the Maritime Museum	
18.00 – 19.30	Maritime Museum Civic Reception with full buffet provided courtesy of the Lord Provost and Aberdeen City Council	

Tuesday 12/05/2026

08.30 – 09.00	Registration	P&J Live
09.00 – 10.00	**Plenary Session Four** Pathology & disease Andy Roe (University of Glasgow)	P&J Live
10.00 – 11.00	**Plenary Session Five** Public Health: Outbreak / Incidence management Steen Ethelberg (Statens Serum Institut)	P&J Live
11.00 – 11.30	Refreshments	P&J Live
11.30 – 12.30	**Plenary Session Six** Pathogen: Biology & Pathogenesis Nicky O'Boyle (Trinity College Dublin)	P&J Live
12.30 – 13.30	Lunch	P&J Live
13.15 – 14.15	Pop Up Session 2 Regulatory Round Table discussion: Pathogenic E. coli – evolving definitions and effect on policy	P&J Live
13.30 – 14.30	**Poster Session 2 – Even numbers**	P&J Live

14.30 – 16.30	**Parallel Session C** Pathogen Microbial ecology Heather Allison (University of Liverpool)	P&J Live
15.30 – 16.00	Refreshments	P&J Live
14.30 – 16.30	**Parallel Session D** Pathogen Detection & Surveillance Claire Jenkins (UKHSA)	P&J Live
16.30 – 17.00	Flash **Poster Session**	P&J Live
19.00 for 19.30	**Conference Dinner and Ceilidh** (Optional - must be purchased in advance online)	Union Kirk

Wednesday 13/05/2026

08.30 – 09.00	Registration	P&J Live
09.00 – 10.00	**Plenary Session Seven** Management of STEC as a One Health pathogen Robert Atterbury (Nottingham University)	P&J Live
10.00 – 11.00	**Roundtable discussion** STEC Genomics Across Countries: Optimising Approaches for Surveillance and Risk Management Jacqui McElhiney (FSS, chair)	P&J Live
1100 – 11.30	Refreshments	P&J Live
11.30 – 12.00	**Plenary Session Eight** VTEC, the way forward David Gally (University of Edinburgh)	P&J Live
12.00 – 12.15	Closing Session	P&J Live
12.30 – 13.45	Packed Lunch	P&J Live
Afternoon	Optional Social Excursion	

Thursday 14/05/2026

There is a Post Symposium Workshop on the Thursday, this is not part of the main conference programme and is only for those who have specifically signed up in advance.

Post-symposium workshop

09.00 – 09.30	Registration	P&J Live
09.30 – 12.15	Morning Sessions	P&J Live
12.15 – 13.15	Lunch	P&J Live
13.15 – 16.15	Afternoon Sessions	P&J Live

General information

Conference venues

Sunday, Monday, Tuesday, Wednesday 10-13 May 2026

Main conference venue

P&J Live, East Burn Road, Stoneywood, Aberdeen AB21 9FX

Accessibility information:

You can find information on accessibility at the venue at P&J Live – Accessibility

In general:

Toilets: there are accessible toilets on all levels

Lift and Escalators: there are lifts and escalators to all levels

Concourse (Ground Floor): Registrations, Catering, Visual Presentations and Exhibition

The View (First Floor): Help Desk, informal seating area, all conference rooms

Taxi rank: Aberdeen Taxis pick-up and drop-off point

Parking: On-site parking is available, pay on exit

No Cash Machine/ATM: in support of P&J Live's green credentials, all the bars, kiosks and restaurants accept card payments only, this is more efficient, safe and ensures a speedy transaction.

WiFi: FREE WiFi is available at the venue. No need for a code, simply connect via the landing page when prompted.

Smoking: P&J Live is a no-smoking building, this includes the use of e-cigarettes and vaping, but they do have specific outside smoking points for those wishing to smoke outside.

For more FAQs regarding the conference venue visit the P&J Live website:

www.pandjlive.com/faq/#visiting-faq

MONDAY 11 MAY 2026

CIVIC RECEPTION, ABERDEEN MARITIME MUSEUM

Aberdeen Maritime Museum is in the city centre, close to the Harbour and easily accessible from throughout the city. The entrance is on Shiprow. The venue is accessible and we will have a team on hand to help if required.

Dress code: Smart-casual.

TUESDAY 12 MAY 2026

Conference Dinner Union Kirk, 333 Union Street, Aberdeen, AB11 6BS

Union Kirk is in the city centre and easily accessible from throughout the city. The entrance is on Bon Accord Street. The venue is accessible and we will have a team on hand to help if required.

Dress code: Smart-casual.

Bus Services

The Jet Service 727 will take you direct from Aberdeen Union Square bus station via P&J Live to Aberdeen Airport. The fleet of Jet 727 buses feature contactless payments, leather seats, free wi-fi and USB charging available onboard.

Departing From	To	First Bus	Last Bus
Union Square	Aberdeen Airport	02:50	23:45
Bucksburn Police Station	Aberdeen Airport	03:09	00:06
P&J Live	Aberdeen Airport	03:15	00:11

www.stagecoachbus.com

How to Pay: Cash (change given), Contactless Card Payments, Mobile tickets with Stagecoach Bus App: www.stagecoachbus.com/promos-and-offers/national/stagecoachbusapp

The Ember Bus Service is a relatively new all-electric bus service. It offers stops at the City Centre, P&J Live, and the University as well as routes to other cities. The fleet of Ember buses feature leather seats, free wi-fi and USB charging available and live tracking of your bus.

How to Pay: Unlike the other local buses, the Ember bus must be booked and reserved at least ten minutes in advance: https://www.ember.to/

Currency and Banks

The official currency of the UK is £ sterling. Commonly accepted credit cards are Visa and Mastercard. Display signs are usually visible in all restaurants and shops indicating which cards they accept.

There is a range of banks in Aberdeen City Centre with ATMs. Most banks are only open until early afternoon on Saturdays and closed all day Sunday. There is no ATM at P&J Live.

Smoking

The Smoking, Health and Social Care (Scotland) Act 2005 made it an offence to smoke, or permit someone else to smoke, within premises which are enclosed or substantially enclosed. All conference venues are no-smoking buildings, this includes the use of e-cigarettes and vaping, but there are specific outside smoking points for those wishing to smoke outside.

Restaurants

A selection of restaurants, eateries and bars can be found on VisitAberdeenshire's website www.visitabdn.com/food-and-drink

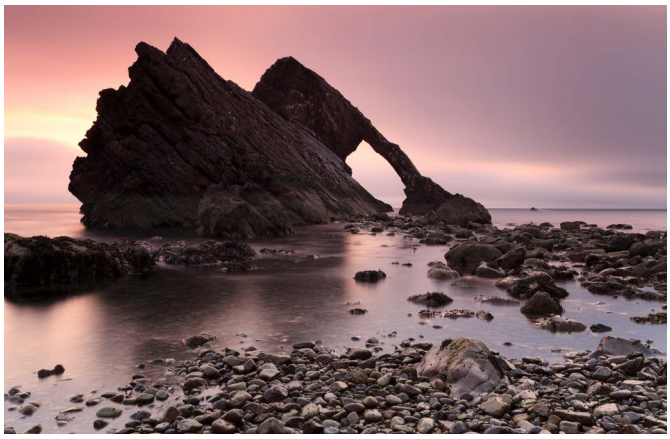
But to help you chose the local's have suggested some of their favourite places to eat and drink:

Restaurants	Pub	Site Seeing
• **Jewel in the Crown** Indian • **The Albyn Bar and Restaurant** • **The Atrium** • **Silver Darling** (noting its pricey but iconic fish restaurant) • **Mi Amore** great value Italian • **Café Bohème** excellent French food • **Muzo's Turkish Kitchen** • **Rishis** amazing Indian Restaurant on George Street • **Rustico** Italian	• **The Howff** • **Under the Hammer** • **Ma Cameron's** • **The Prince of Wales** very traditional Scottish pub • **The Grill** the most traditional pub in Aberdeen – range of whisky. • **O'Malley's** new Irish bar on Crown Street • **The Globe** • **The Noose and Monkey**	• **Grampian Transport Museum (Alford)** • **Aberdeen Beach** especially Don Mouth • **Seal watching at Newburgh** but you need to watch where you go as some areas are prohibited to the public • **Greyhope Bay** great coffee shop with dolphin and seal watching • **Balmedie Beach** • **Old Deeside Railway path** • **King's college , Old Aberdeen and Botanic Gardens** • **Marischall College** • **Duthie Park Glasshouse Winter Gardens** • **Gordon Highlanders Museum** • **Open top bus tours of Aberdeen City**

Extending your stay

If you are keen to extend your stay and see what Aberdeenshire and the rest of Scotland has to offer, you can find more information at

www.visitscotland.com and www.visitabdn.com



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With thanks to our Sponsors, Exhibitors and those who gave Grants to support VTEC 2026

Platinum Sponsors

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THE FOOD SAFETY LAW FIRM

Food safety lawyers and experts since 1993

For 30 years, Marler Clark has been the leading expert in food safety law and has represented thousands of victims in food safety cases against large corporations including Walmart, Dole, McDonald's, and Nestle. Our team is dedicated to helping victims of foodborne illness outbreaks

The food attorneys at Marler Clark, Inc., PS are the USA's leading lawyers representing victims of foodborne illnesses such as E. coli, Salmonella, and Listeria.

Marler Clark was formed in 1998 following the historic Jack-in-the-Box E. coli outbreak that sickened nearly 700, hospitalizing hundreds and causing the death of 4 children. Bill Marler and Andy Weisbecker, who represented the E. coli victims, joined forces with Bruce Clark and Denis Stearns, who represented Jack-in-the-Box, to form the first and only law firm with its sole mission to represent the victims of foodborne illnesses like E. coli, Salmonella, Listeria, Hepatitis A, and others. Since 1998, Marler Clark has been involved in all major outbreaks in the United States and has consulted on cases worldwide. Together, they have secured verdicts and settlements for their clients amounting to more than $850,00,000.

https://marlerclark.com/

BioMérieux - Silver Sponsor

A family-owned company, bioMérieux has grown into a global leader in in vitro diagnostics, driven for over a century by a strong entrepreneurial spirit and a constant commitment to improving public health worldwide. Since 1963, bioMérieux has played a key role in advancing diagnostics to support better health outcomes. For more than 30 years, the company has also supported the food industry with innovative solutions that help ensure food safety and quality. Through its Augmented Diagnostics approach, combining advanced microbiology and molecular technologies with data science and genomics, bioMérieux empowers industry players to make confident, data driven decisions across the entire production value chain.

https://www.biomerieux.com/corp/en/our-offer/food-safety-and-quality.html

Hygiena - Silver Sponsor

Hygiena delivers rapid microbial detection, monitoring, and identification solutions to a wide range of industries, including food and beverage, healthcare, hospitality, pharmaceuticals, and personal care. Utilizing advanced technologies and patented designs, Hygiena provides industry-leading ATP monitoring systems, PCR-based pathogen and non-pathogen detection and characterization systems, allergen tests, environmental collection devices, and more. Hygiena is committed to the mission of providing customers with high-quality, innovative technologies that are easy-to-use and reliable, backed by excellent customer service and support. Headquartered in Camarillo, California, with multiple offices around the world, and over 180 distributors in more than 100 countries worldwide, Hygiena-branded products span the globe.

https://www.hygiena.com/food-safety/pathogen-detection/stec

Exhibitors

Seropsep

SEROSEP https://www.serosep.com/

SRUC School of Veterinary Medicine and Biosciences

SRUC SCHOOL OF VETERINARY MEDICINE AND BIOSCIENCES https://www.sruc.ac.uk/study-with-us/school-of-veterinary-medicine-and-biosciences/

Mast PLC

MAST Mast Group https://www.mast-group.com/uk/about/company-details/

Workshop Sponsors

The workshop was supported by The Food Safety Research Network, Quadram Institute

FOOD SAFETY RESEARCH NETWORK https://fsrn.quadram.ac.uk/

The workshop was supported by bioMérieux

BIOMÉRIEUX https://www.biomerieux.com/corp/en.html

Poster Prize Sponsorship

The ASM journal, Animal Microbiology, supported three poster prizes.

AMERICAN SOCIETY FOR MICROBIOLOGY https://journals.asm.org/journal/asm-animal-microbiology

The FEMS Microbiology Letters journal supported a poster prize and a Thematic Issus on VTEC / STEC.

https://fems-microbiology.org/about_fems/network-and-activities/journals/fems-microbiology-letters/

GRANT FUNDERS:

FEMS A FEMS Organiser Grant was award by the Federation of Microbiology Societies (FEMS).
https://fems-microbiology.org/about_fems/network-and-activities/grants/

Food Standards Scotland supported the main conference and post-symposium workshop with a grant.
https://www.foodstandards.gov.scot/

MICROBIOLOGY SOCIETY The Microbiology Society supported invited speakers costs.
https://microbiologysociety.org/

Applied Microbiology International The Applied Microbiology International society supported a one-year annual membership for the delegates.
https://appliedmicrobiology.org/

Keynote Biographies

Keynote Biographies

Dr. Ana Allende

Senior Researcher Cebas-csic

Dr. Ana Allende from CEBAS-CSIC (Spanish National Research Council) in Spain is a Research Professor with focus on safety of fresh produce and water. She holds several positions in (inter)-national institutions including Chair of the BIOHAZ panel at the European Food Safety Authority (EFSA), coordinator of health risks in the CSIC emergency committee, Member of the Joint FAO/WHO Expert Meetings on Microbiological Risk Assessment (JEMRA) and member of the Advisory Committee of Directors from the CSIC Vice-rectorate for Research and Innovation. From 2016 to 2025 she exerted as vice-director of the CEBAS-CSIC. Since 2018, she has participated in a total of 8 WG organized by the JEMRA. She has built up more than twenty years of scientific research but also management experience by executing, initiating and guiding international and national research projects in the area of microbial safety of fresh produce and water. She has been the principal investigator of national (10) and international (17) projects as well as technology transfer contracts with private companies (35). In her role as Principal Investigator at CEBAS-CSIC she leaded research projects for a total value of €4,900,000. On the other hand, she has leaded technology transfer contracts with private companies for a total value of €3,300.000. She is the author of more than 266 research articles published in international journals included in the Scientific Citation Index (SCI) on food safety and more particularly in the safety of fruits and vegetables as well as intervention strategies able to control microbial contamination of fresh produce and water, with a particular emphasis in water disinfection. H-index = 64. Her scientific production also includes more than 95 scientific opinions published by the BIOHAZ panel of EFSA. Promotor of 8 PhD students (past and present). She has been invited as speaker in more than 70 international conferences and workshops and about 50 national congresses and workshops. Participates in the PhD Program at the University of Murcia and in the One Health Master's Program at the Autonomous University of Barcelona (2022–2025). She has acted as an Expert Evaluator in the Horizon Europe Program in 8 panels. Dr. Allende is collaborating with National and International authorities to monitor the transmission of emerging pathogens within the population using wastewater-based epidemiology (WBE) as an early warming tool for COVID-19. The work developed since 2020 in WBE was central to receiving the CSIC Award for Knowledge Transfer and Entrepreneurship in 2024.

Prof. Heather Allison

Professor of Molecular Microbiology, University of Liverpool

www.liverpool.ac.uk/people/heather-allison

Dr Robert Atterbury

Associate Professor, University of Nottingham

Dr. Robert Atterbury is an Associate Professor in Microbiology at the School of Veterinary Medicine and Science, University of Nottingham. He leads a research group studying antimicrobial resistance in zoonotic bacterial pathogens, and how they can be controlled without the use of conventional antibiotics. A key focus of this work is the biology and therapeutic potential of bacteriophages and predatory bacteria such as Bdellovibrio bacteriovorus. Dr. Atterbury obtained his BSc in Biology, PhD in Microbiology, and MRes in Bioinformatics from the University of Nottingham. His doctoral research investigated bacteriophage control of Campylobacter jejuni in poultry. Following this, he undertook postdoctoral work at the University of Bristol with Prof Paul Barrow and Dr. Viv Allen in a range of EU, UKRI and UK Government-funded projects on the epidemiology and control of Campylobacter and Salmonella in poultry. Returning to Nottingham as a Research Fellow in Prof. Liz Sockett's group, Dr. Atterbury performed pioneering work on using the predatory bacterium Bdellovibrio bacteriovorus - developing the first successful therapeutic model in vertebrates. In 2010, he joined the School of Veterinary Medicine and Science as a Lecturer, where he established a multidisciplinary research group investigating phage biology, epidemiology of zoonotic pathogens, and applications of phages across the food chain. His research projects include the use of phage to control Avian Pathogenic E. coli, funded by the European Union, and the investigation of antimicrobial resistance transmission in E. coli between human, animal and environmental sources in the UK and India (jointly funded by the BBSRC and Government of India). He has also received funding to study the biocontrol of Vibrio cholerae (British Council funded) and antimicrobial resistance in, and biological control of Pseudomonas aeruginosa (BBSRC and industrial funded).

Dr Sooria Balasegaram

Consultant Epidemiology, UK Health Security Agency

Sooria Balasegaram has been working in public health for almost 30 years. She was a medical Consultant in Communicable Disease Control in London from 2006-2010, and since then, as a consultant epidemiologist at UK Health Security Agency (UKHSA) Field Services covering the South East and London regions of England. She is the current UKHSA theme lead for Surveillance in the Health Protection Research Unit for Gastrointestinal Infections. During her time at UKHSA, she has worked on and chaired the development of the English Public Health STEC guidance and the Enteric Fever Guidance. She has worked with the UKHSA national gastrointestinal infections team on outbreaks and surveillance of a range of gastrointestinal pathogens, and acted as the Lead Epidemiologist for STEC for England between 2022 and 2024. She has led on the epidemiological investigation and management of numerous outbreaks, and surveillance and research projects in gastrointestinal infections particularly in STEC. Sooria is a supervisor for the UK Field Epidemiology Training Programme and has previously been a coordinator and supervisor for the European Programme for Interventional Epidemiology Training and the European Public Health Microbiology Training Programme.

Dr Paula Coccia

Consultant Paediatric Nephrologist, Hospital Italiano De Buenos Aires

Paula Alejandra Coccia is a Professor of Pediatrics at the Hospital Italiano de Buenos Aires University, School of Medicine, and a Consultant Paediatric Nephrologist in the Division of Pediatric Nephrology, Department of Pediatrics, Hospital Italiano de Buenos Aires, Argentina. She graduated as a medical doctor from the University of Buenos Aires in 1991 and completed her clinical training in Pediatric Nephrology at the Hospital de Niños Ricardo Gutiérrez in Buenos Aires. She has been a member of the Pediatric Nephrology team at the Hospital Italiano de Buenos Aires since 2003. Dr Coccia currently serves as Coordinator of the Pediatric Renal Transplant Unit, providing specialised care to children with advanced kidney disease and those requiring renal transplantation, as well as long-term post-transplant follow-up. In the academic setting, she is Director of the Pediatric Nephrology Fellowship Program at the University of Buenos Aires, based at the Hospital Italiano. She is also an Assistant Professor of Pediatrics at the Hospital Italiano de Buenos Aires University, School of Medicine, where she is actively involved in undergraduate and postgraduate teaching, and in the supervision of residents and fellows. Her research activities are closely linked to national and multicentre collaborative studies conducted through the Pediatric Nephrology Committee of the Argentine Society of Pediatrics. Her main research interests include Shiga toxin–producing Escherichia coli–associated haemolytic uraemic syndrome (STEC-HUS), atypical haemolytic uraemic syndrome, complement-mediated renal diseases, chronic kidney disease in children, and pediatric renal transplantation. She has participated in and led research projects aimed at improving early diagnosis, therapeutic strategies, and long-term outcomes in these conditions.

Dr Lauren Cowley

Senior Lecturer, University of Bath

Dr. Lauren Cowley is a senior lecturer in microbial genomics and bioinformatics in the Milner Centre for Evolution at the University of Bath. Lauren joined the university as prize fellow of bioinformatics in 2018, progressed to lecturer in 2022 and senior lecturer in 2023. Lauren's background is in public health and genomic epidemiology. She has worked on several international outbreaks, including implementing real-time genomic surveillance during the West Africa Ebola outbreak and working as an embedded scientist on the COVID-19 Taskforce in the UK Cabinet Office. She also previously worked at Public Health England (now UKHSA) in the Gastrointestinal Bacteria Reference Unit. Her research centres around genomic epidemiology, GWAS and machine learning for microbial genomics. She has a special interest in Shiga-toxigenic Escherichia coli since her PhD on the genomic basis of phage typing of STEC O157.

Prof Steen Ethelberg

Head of Section, Statens Serum Institut

I'm Steen Ethelberg, an epidemiologist with the Statens Serum Institut, in Copenhagen, Denmark. There I work as a senior scientist in epidemiology and am head of Section for Zoonotic, Food and Waterborne Infections at the Department of Epidemiology. We are responsible for surveillance, epidemiological analyses and outbreak investigations within the area of food- and waterborne and zoonotic infections in Denmark. We're also the host site for the European field epidemiology training program, EPIET, and I'm the EPIET supervisor for Denmark. I'm is also a professor at the Global Health Department at University of Copenhagen. My research interest concern disease surveillance methods and the epidemiology of gastro-intestinal infectious diseases, including STEC/VTEC, with the aim of, hopefully, obtaining better prevention hereof.

Prof David Gally

Professor of Microbial Genetics, University of Edinburgh

I hold a personal chair in Microbial Genetics at the University of Edinburgh and have been part of the Roslin Institute since 2011. I led the BBSRC Institute Strategic Programme on the 'Control of Infectious Diseases' in Livestock (2017-2023) at the Roslin Institute. I was also seconded to Food Standards Scotland as their Chief Scientific Advisor from 2020-24. My background is in Microbiology, initially bacterial physiology for my PhD and first Post Doctoral position at the University of Michigan (cell wall assembly) but I then moved into gene regulation during a second Post Doctoral post in North Carolina and then returned to the UK supported by an MRC Career Development Fellowship which was focused on the regulation of fimbrial adhesins in E. coli. I obtained a Lectureship in Bacteriology at Edinburgh Vet School in 1998 which soon led to a DEFRA Veterinary Fellowship on the biology of enterohaemorrhagic E. coli (EHEC) which has remained an important research focus of my group's research for nearly twenty years. My research is focused on the evolution of bacterial pathogens, with a specific interest on infections caused by E. coli. I collaborate extensively and my local group combines bioinformatics and wet science to investigate basic biological concepts, including host specificity. Key current areas are: (1) E. coli genomic variation accounting for differences in the severity of disease and zoonotic potential. (2) Machine-learning methods to predict zoonotic potential. (3) Predictive phage therapy of E. coli urinary tract infections and STEC in cattle. (4) AMR transmission through the food chain. (5) Transcriptional and post-transcriptional mechanisms of E. coli colonisation factor expression. (6) Vaccine and adjuvant development in ruminants, in particular based on the use of flagella.

Prof George Gunn

Emeritus Professor Population Medicine And Zoonoses, SRUC

Professor George Gunn is currently Emeritus Professor at Scotland's Rural College in Inverness (SRUC), most recently Director of the ambitious SRUC Rural and Veterinary Innovation Centre (RAVIC) developed in Inverness as part of SRUC's new Veterinary School. His range of expertise includes livestock disease control with an overarching interest in population medicine and the dynamics of infectious inter-herd diseases and zoonoses. He created the SRUC research team in Inverness (Epidemiology Research Unit), later becoming Head of Veterinary Epidemiology as Professor of Population Medicine and Zoonoses. He helped win over £50 million for SRUC and collaborating Institutions. His primary skills are in applied medicine and epidemiological science, with an objective of sustaining rural communities through such work as RAVIC. George has acted as lead for many research projects both in Scotland, across UK and Internationally and has many research publications and reports, delivering applied research, with presentations around the world. Examples of multi-disciplinary, multi-institutional project management include being Director of the EPIC partnership which advises the Scottish Government on animal disease outbreaks. He was also part of the management teams for the concluding EC BVD Control and ParaTB Tools Projects and was part of the Defra Surveillance and TB Reviews. He has extensive experience working with industry and helped initiate several cattle health programmes, including HI-Health, the Scottish and UK Pig Health and Cattle Health Schemes and helped initiate and underpin Cattle Health Certification Standards (CHeCS). Working with many other important actors, his applied research has formed the basis of several high scoring REF Impact Case Studies for SRUC /University of Edinburgh. Since STEC became an issue for Scotland George became the epidemiologist responsible for running large-scale field studies that helped define STEC epidemiology and the extent of the public health threat from the livestock sector in Scotland. An integral part of the pivotal Wellcome funded International Partnership Research Awards in Veterinary Epidemiology (IPRAVE) he helped establish and became responsible for the category 3 laboratory now within RAVIC. His work underpinned many highly collaborative research publications that remain relevant to VTEC 2026 such as "Chase-Topping, M, Dallman, TJ, Allison, L, Lupolova, N, Matthews, L, Mitchell, S, Banks, CJ, Prentice, J, Brown, H, Tongue, S, Henry, M, Evans, J, Gunn, G, Hoyle, D, McNeilly, TN, Fitzgerald, S, Smith-Palmer, A, Shaaban, S, Holmes, A, Hanson, M, Woolhouse, M, Didelot, X, Jenkins, C & Gally, DL 2023, 'Analysis of Escherichia coli O157 strains in cattle and humans between Scotland and England & Wales: implications for human health', Microbial Genomics, vol. 9, no. 9, 001090. doi.org/10.1099/mgen.0.001090"

Dr Claire Jenkins

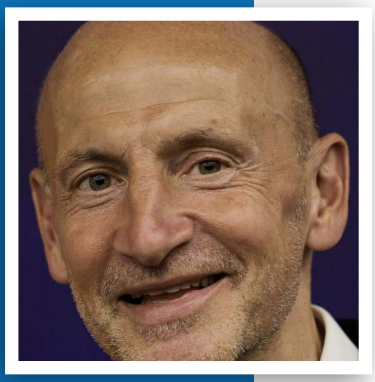
Clinical Scientist, UKHSA

I started working for UK Health Security Agency, then the Public Health Laboratory Service, as a Clinical Scientist in 1996. I became head of the E. coli Reference Laboratory in 2012 and deputy head of the Gastrointestinal Bacteria Reference Unit in 2014, the same year as we implemented whole genome sequencing as our routine typing method for surveillance and outbreak investigation.

Dr Sally Johnson

Consultant Paediatric Nephrologis, Newcastle Upon Tyne Hospitals Nhs Foundation Trust

Dr Sally Johnson is a consultant paediatric nephrologist at the Great North Children's Hospital (Newcastle, UK) and the lead paediatric clinician at the National Renal Complement Therapeutics Centre (based in Newcastle, UK), which has a national role in the management of patients with the rare kidney diseases atypical HUS (aHUS) and C3 glomerulopathy (C3G). She undertook clinical and academic training in the West Midlands, including a PhD in aHUS. She was Chief Investigator of The National Study of MPGN and C3G and of the ECUSTEC trial, an RCT of Eculizumab in STEC-HUS, and co-applicant for the Stopping Eculizumab Safely in aHUS trial. She was Research Secretary for the British Association for Paediatric Nephrology (BAPN) (2019-2022) and chair of the BAPN Clinical Studies Group until 2024. She is chair of the UK Kidney Association STEC-HUS Rare Disease Group. She is a member of the Kidney Research UK grants committee. She is a late discoverer of running and has run five half-marathons and the London marathon, raising money for research into kidney disease.



Mr Alec Kyriakides

Independent Food Safety Consultant

Alec Kyriakides is a food safety consultant with over 35 years experience in the industry. In his 28 years with the retailer Sainsbury's, Alec managed safety, quality, supplier performance, technical training, serious incidents, customer complaints, analytical assurance and the in-house accredited laboratory. Prior to Sainsbury's, he worked in food manufacturing including the dairy and brewing industries. He is the co-author of books on the practical control of food borne pathogens including Salmonella, Listeria, C. botulinum, Campylobacter and E. coli. Alec has sat on a number of influential industry and government committees and is currently Chair of the Advisory Committee on the Microbiological Safety of Food. He is a Non Executive Board Director of Campden BRI, a Trustee of the Institute of Food Science and Technology (IFST), Chair of the Safe to Trade Technical Standards Committee and Chair of the FSA Root Cause Analysis Steering Committee. Alec is an Honorary Lecturer at Queen's University Belfast.

Mr Stefano Morabito

Research Director, Istituto Superiore di Sanità

Stefano Morabito, MSc (Biology). Director of the Food borne Diseases Unit within the Department of Food Safety, Nutrition, and Veterinary Public Health at the Italian National Institute of Health. Serves as Director of the European Union Reference Laboratory for E. coli in the food and feed sector and Co-Director of the European Union Reference Laboratory for Food- and Water-borne Bacteria in the Public Health sector. Leads research projects on the molecular basis of virulence in Shiga toxin-producing E. coli and other pathogenic E. coli strains, as well as the ARIES research infrastructure dedicated to applying genomics to public health microbiology and high-intensity data analysis. Author of numerous peer-reviewed publications on the epidemiology of STEC infections, their circulation in animal reservoirs and the environment, and the evolution of virulence in these microorganisms. Edited the book Pathogenic Escherichia coli: Molecular and Cellular Microbiology, published by Caister Academic Press. Coordinates the IRIDA-ARIES platform, designed for data collection and analysis to enhance genomic surveillance of human infections caused by food borne pathogens, including STEC. His work focuses on promoting awareness of the One Health approach to address food borne infections and supporting the transition toward a sustainable and equitable food system in the medium to long term.

Dr Nicky O'Boyle

Assistant Professor, Trinity College Dublin

Dr Nicky O'Boyle is an Assistant Professor of Microbiology at Trinity College Dublin. His PhD with Dr Aoife Boyd at the University of Galway focussed on mechanisms of cellular adhesion in the food borne pathogen Vibrio parahaemolyticus. He then moved to The University of Glasgow in 2014 where he worked in the laboratory of Dr Robert Davies, developing advanced respiratory cell culture models to study infection dynamics of the ruminant pathogen Mannheimia haemolytica. In 2017 again at The University of Glasgow, Dr O'Boyle joined Professor Andrew Roe's Group where he studied diverse strains of Escherichia coli associated with distinct disease outcomes in humans. His independent research group was established in 2022 at University College Cork before relocating to Trinity College Dublin in 2024. Their work encompasses investigating the fundamental molecular biology of bacterial pathogens, and the development of novel therapeutic strategies to combat bacterial disease. Dr O'Boyle's group have a strong focus on Gram-negative enteric pathogens including enterohaemorrhagic E. coli, Crohn's disease-associated pathobionts, and uropathogenic E. coli. His group aim to exploit insights into niche-specific pathogen expression patterns to develop precision anti-virulence strategies.

Prof Andrew Roe

Professor of Molecular Microbiology, University of Glasgow

Dr Nicky O'Boyle is an Assistant Professor of Microbiology at Trinity College Dublin. His PhD with Dr Aoife Boyd at the University of Galway focussed on mechanisms of cellular adhesion in the food borne pathogen Vibrio parahaemolyticus. He then moved to The University of Glasgow in 2014 where he worked in the laboratory of Dr Robert Davies, developing advanced respiratory cell culture models to study infection dynamics of the ruminant pathogen Mannheimia haemolytica. In 2017 again at The University of Glasgow, Dr O'Boyle joined Professor Andrew Roe's Group where he studied diverse strains of Escherichia coli associated with distinct disease outcomes in humans. His independent research group was established in 2022 at University College Cork before relocating to Trinity College Dublin in 2024. Their work encompasses investigating the fundamental molecular biology of bacterial pathogens, and the development of novel therapeutic strategies to combat bacterial disease. Dr O'Boyle's group have a strong focus on Gram-negative enteric pathogens including enterohaemorrhagic E. coli, Crohn's disease-associated pathobionts, and uropathogenic E. coli. His group aim to exploit insights into niche-specific pathogen expression patterns to develop precision anti-virulence strategies.

Abstracts

Keynotes

STEC IN SCOTLAND

Professor George J Gunn
SRUC

Why STEC Still Matters: Insights, Impact, and the Imperative for Action

Dr Sally Johnson[1]

[1] Newcastle Upon Tyne Hospitals NHS Foundation Trust

In the decades that have passed since VTEC (STEC) was first recognised as a major cause of haemolytic uremic syndrome, there have been considerable advances in the understanding of the pathogenesis of STEC-HUS.

However, STEC-HUS remains a considerable cause of mortality and morbidity well into the 21st century and there is still no definitive treatment to reverse the condition once it is established. Improvements in survival and reduction in morbidity have mainly come about through progress in the provision of supportive and critical care.

This talk will highlight the clinical challenges in improving the outcome for STEC-HUS, and will include perspectives from patients and carers with lived experience of STEC-HUS about the long-term impact of the condition, emphasising that the need for a collaborative “One Health” approach to reducing morbidity and mortality from STEC is greater than ever.

Global pathogens require a holistic approach to imagine solutions: Shiga toxin-producing E. coli and the One Health paradigm

Stefano Morabito[1]

[1] Istituto Superiore di Sanità

STEC is a zoonotic pathogen that has come to the attention of the scientific communities, the health services, the food production sector and the civil society due to the severity of the infections it causes, the complexity of exposure routes, and the media coverage it receives. The most severe forms of infection in humans occur mainly in the most vulnerable groups, such as children and the elderly, with implications for the care provided by families and the healthcare system. Although the nature of the transmission of the infection from the natural reservoir to humans is primarily food-borne, with food of animal origin at the apex, increasing attention is being paid to transmission routes linked to the environment, such as, consuming vegetables commodities, swimming in recreational waters, direct contact with infected animals and interpersonal contact. Due to the complexity of human exposure pathways to infections, a thorough understanding is required of the circulation of these pathogenic microorganisms in animal reservoirs, food vehicles and the environment, which is increasingly exposed to contamination, including through circular economy practices aimed at reusing biomass and reclaimed water to restore soil fertility and the efficiency of intensive farming systems.

Understanding the circulation of STEC strains therefore requires the integration of knowledge and analytical approaches from different sectors – human, animal, food and environmental – within a One Health framework. Of great importance is also the study of the impact of STEC infections on civil society through the dissemination of information via the media, and social media in particular, which can convey a misleading narrative regarding the causes of infections and the risks associated with the consumption of certain food products by vulnerable groups.

The involvement of civil society, ultimately, acts as the link between science and policy, having the power of applying pressure to the policy makers to deploy effective control measures to mitigate human exposure and the impact of infections. Therefore, it is crucial to disseminate the knowledge acquired and the measures developed in ways and using language accessible to a non-specialist audience, avoiding sensationalism and underestimation of risks, with the aim of completing the One Health cycle, which represents the best possible strategy for the management of STEC infections.

Exploring the prophage profile of STECs using 'AllTheBacteria' ever sequenced

Dr Lauren Cowley[1]

[1] University Of Bath

Shiga-toxigenic Escherichia coli is famous for its vast prophage composition that can contribute up to 20% of their genomes. These phages have significant importance, not only for carrying their defining Shiga toxin virulence genes but also as time capsules from when those strains might have encountered particular phage. Deciphering which strains carry which phage profile has previously been difficult, but due to recent advances in indexing and searching bioinformatics tools, we are now able to catalogue this on a wider scale. We make use of the AllTheBacteria database and Lexicmap software to query prophage regions from long read sequenced STEC strains against all publicly available bacterial sequences on the sequence read archive (2.4M strains). Results demonstrate how interconnected STEC is with other pathotypes of E. coli. We also use DefenseFinder to match up prophage profile determination with host phage defense system profiles. We hope this work will further advance our knowledge on the role of phage-host interactions in shaping the emergence and evolution of STECs as well as wider diverse E. coli.

Food Safety - Lessons from the past and Current challenges

Mr Alec Kyriakides[1]

[1] Independent Food Safety Consultant

Over thirty years have passed since the large VTEC outbreak caused by beef burgers from a fast food outlet in the USA. Our understanding of the organism, its sources and vehicles of transmission to foods and humans have helped inform the control of the organism and risk management strategies. This presentation looks at outbreaks and explores the lessons the food industry has learnt and how this has influenced current control strategies. It also examines the challenges the industry continues to face including consideration of the gaps that prevent consistently effective control. This will encompass matters such as prevalence, survival, growth, analysis as well as risk considerations in relation to developments in food production such as vertically farmed produce.

Management of STEC HUS in 2026: now and looking to the future

Dr Paula Coccia[1]

[1] Hospital Italiano de Buenos Aires

This keynote will focus on current and emerging interventions aimed at preventing and treating Shiga toxin–producing Escherichia coli–associated hemolytic uremic syndrome (STEC-HUS). A central theme of the session will be the real-world difficulty of early identification of STEC infection, which remains a major barrier to timely intervention despite expanding therapeutic options.

We will highlight frontline diagnostic challenges, early clinical warning signs, and the impact of delayed recognition on outcomes — particularly relevant for clinicians directly involved in acute patient care.

The talk will review several potential and evolving therapeutic strategies, including early and optimized hydration, complement blockade in selected scenarios, bacteriostatic antibiotics, and toxin-neutralizing antibody approaches. These options will be discussed within a practical clinical framework, with particular attention to the current state of evidence, remaining uncertainties, and where we stand today regarding their role in routine care.

Environmental stressors as dual drivers of die-off, persistence, and detectability of enteric pathogens on leafy greens

Dr. Ana Allende[1]

[1] CEBAS-CSIC

Preharvest contamination of leafy greens is commonly assessed using culture-based methods. A substantial body of research has been conducted to quantify population-level die-off following exposure to environmental stressors. However, evidence indicates that these stressors not only drive pathogen inactivation but also promote physiological states that challenge both detectability and risk interpretation. Environmental conditions can therefore simultaneously govern die-off, persistence, and culturability of enteric pathogens on leafy greens.

Field studies conducted demonstrate that weather variables such as solar radiation, relative humidity, temperature, wind speed, and dew point strongly influence biphasic survival patterns of Escherichia coli and Salmonella enterica following irrigation with contaminated water. While these conditions accelerate overall population decline, they consistently generate stable tail populations that cannot be explained by uniform inactivation alone. Mathematical modelling of these dynamics links specific stressors, particularly high solar radiation and limited water availability, to increased switching into a persister state in the phyllosphere. In parallel, evidence from molecular monitoring approaches shows that under comparable environmental stress, culturable cells may fall below detection limits while viable but non-culturable (VBNC) cells remain detectable using viability-qPCR, both in irrigation water and on fresh produce.

These findings indicate that failure to recover culturable bacteria does not necessarily imply microbial absence, but may instead reflect stress-induced physiological transitions. Environmental conditions that appear to reduce microbial loads may concurrently enrich for viable subpopulations with enhanced tolerance to subsequent stresses and reduced detectability. Integrating persistence and VBNC states into exposure assessment is essential for accurately evaluating food safety risks.

Aurodox: Disarming Pathogens through Type III Secretion Inhibition

Prof Andrew Roe[1], Dr Rebecca McHugh[1]

[1] University Of Glasgow

Aurodox, a polyketide antibiotic produced by Streptomyces goldiniensis, has emerged as a promising anti-virulence compound targeting the bacterial Type III secretion system (T3SS). Our research has defined its mechanism, biosynthetic origins, and translational potential in infection control. We first established that aurodox selectively inhibits T3SS-dependent effector secretion in pathogenic Escherichia coli and other Gram-negative pathogens without affecting bacterial viability, offering an antivirulence approach that minimizes selection pressure for resistance. Genomic and biochemical analyses of the producing strain revealed the complete aurodox biosynthetic gene cluster, enabling insights into its assembly and potential for engineering derivative compounds. Building on these foundations, we demonstrated that aurodox's inhibitory activity extends across multiple clinically relevant T3SS-utilizing pathogens, including Pseudomonas aeruginosa and Salmonella enterica, highlighting broad-spectrum applicability. Most recently, preclinical studies in a murine model of Shiga toxin–producing E. coli infection showed that aurodox treatment significantly reduced disease severity and toxin-mediated pathology, supporting its therapeutic promise. Together, these studies establish aurodox as a prototype for anti-virulence therapeutics: a natural product repurposed from traditional antibiotic origins to disarm pathogens rather than kill them. This seminar will trace the scientific journey from discovery to in vivo validation, discussing the molecular mechanisms of T3SS inhibition, biosynthetic pathway elucidation, and translational implications for next-generation antimicrobial strategies.

Detecting and managing STEC outbreaks: lessons from Denmark and beyond

Prof Steen Ethelberg[1]

[1] Statens Serum Institut

STEC has repeatedly caused outbreaks ranging from local outbreaks to large multinational events, with substantial variation in severity, transmission routes and microbiological characteristics. A few outbreaks have developed into major public health crises and have reshaped thinking on surveillance, outbreak detection and control. This presentation will highlight key international STEC outbreaks and how the profile of outbreaks has changed over time, including increasing complexity in vehicles, geography and microbiological diversity. Against this background, I will present the Danish experience with STEC outbreak detection, investigation and control, focusing on the organisational structures required for timely action. Effective management depends not only on laboratory methods and surveillance systems, but also on close collaboration between epidemiology, clinical microbiology, food safety authorities and other sectors. Drawing on lessons from STEC and other enteric pathogens, I will discuss how public health set-ups can be organised to assist early outbreak recognition and strengthen response to future events.

Chiral enantiomers of serine repress the enterohaemorrhagic E. coli type three secretion system via modulation of the nitrogen stress response

Emily Addington, Kabo Wale, Emily Horsburgh, Patricia Rimbi, David Mark, Sofia Sandalli, Ester Serrano, Gavin Blackburn, Clément Regnault, Philip Whitfield, James Connolly, Andrew Roe, Dr Nicky O'Boyle[1]

[1] Trinity College Dublin

Bacterial pathogens exploit intricate sensory mechanisms to precisely modulate gene expression in response to host-associated cues. This optimises within-host fitness. D-serine, a host-produced metabolite enriched in urine is toxic to enterohaemorrhagic E. coli, inhibiting its type 3 secretion system (T3SS) and activating the SOS response. While this has been proposed to restrict EHEC to its preferred intestinal niche, the molecular mechanism of virulence repression by D-serine remains incomplete. Here we show that multiple amino acids, including L-serine, converge on this same regulatory pathway and repress the T3SS but without triggering the SOS response. A combination of transcriptomics, metabolomics, and targeted deletions reveal that this regulation is mediated by the release of ammonia, the nitrogenous product of serine breakdown, rather than by sensing of intact serine. Interestingly, despite evolutionary loss of canonical D-serine metabolic capacity, EHEC shows a novel oxidative deamination activity capable of producing this regulatory signal. Deamination has the effect of switching off the nitrogen stress response in T3SS-inducing media as seen by repression of the entire regulon of NtrC – the nitrogen stress response master regulator. Deletion mutants lacking ntrC were unable to fully activate the T3SS and showed no repression of T3SS genes by L- or D-serine. Our data suggest that distal intestinal colonisation by EHEC is facilitated by adaptation of virulence factor regulation to amino acid-depleted environments. This work highlights the crucial interplay between stress responses, metabolism and virulence in an important bacterial pathogen.

Shiga toxin-encoding bacteriophages – more than toxin distribution units

Prof. Heather Allinson[1]

[1] University of Liverpool

Shortly after the first outbreak of E. coli O157:H7 in Traverse City, Michigan in 1982, the strain responsible for causing the outbreak was found to harbour two phages that each encoded Shiga toxin. This led to a rash of work uncovering that bacteriophages were behind the spread and dissemination of the many variants of Shiga toxin across a wide array of E. coli serogroups and other related genera. These Stx phages were normally found to be temperate, lambdoid phages, and the toxin production was linked to the lytic replication cycle of the phage. An explosion of Stx phage sequences was entered into the public databases, and it became clear that Stx phages are not required to be similar to each other, and exist as genomic mosaics of many phages that carry the stx operon. We have used various molecular techniques to study one particular Stx phage, ϕ24$_B$, that was isolated from an E. coli O157:H7 outbreak strain in 1996 in Lanarkshire, Scotland. These studies led to several unusual findings: and1) some Stx phages can superinfect a host cell, despite a functioning immunity region; 2) Stx expression can be limited by exceeding the translational threshold of a bacterial cell; 3) Short tailed Stx phages utilise a highly conserved and essential outer membrane protein, BamA as a host recognition receptor; and 4) a variety of DNA binding proteins carried on the Stx phage impact the expression of bacterial host genes and phenotypes. RNASeq, Nanostring nCounter®, and expression vector cloning have been used to begin to pull apart previously undescribed host cell responses to prophage carriage and prophage control.

STEC diagnostics: Past, Present & Future

Dr. Claire Jenkins[1]

[1] UKHSA

Although Theodor Escherich recognised *E. coli* as a cause of acute infectious diarrhoea in infants as early as 1885, it was nearly 60 years later before enteropathogenic E. coli was isolated from faeces by John Bray in 1945, using antiserum raised in his pet rabbit Snowy, to the E. coli cultured from symptomatic children. However, this method was both resource and labour intensive, and it became obsolete during the latter part of the 20th century as outbreaks of infantile diarrhoea decreased.

Due to the association with severe clinical outcomes, including haemolytic uraemic syndrome (HUS), the emergence of STEC in the 1980s sparked renewed interested in the detection of pathogenic *E. coli* from faecal specimens. Although it was recognised that there were many different serotypes of STEC, STEC O157:H7 was found to be the most common cause of outbreaks and of HUS. As a result, most public health institutions focused their national surveillance systems on detecting STEC O157:H7, using selective media called cefixime tellurite sorbitol MacConkey (CT-SMAC) agar developed specifically to detect this serotype.

The dawn of the molecular era provided scope for the development of PCR assays targeting the Shiga toxin genes (stx), the defining characteristic of STEC group, and therefore the potential to detect all types of STEC in one assay. However, culture of non-O157 STEC has remained problematic. In the UK reference laboratories, and elsewhere, faecal specimens testing positive for STEC using PCR are cultured on semi-selective and non-selective agar. Individual colonies are re-tested by PCR to pinpoint those harbouring stx, thus identifying the STEC isolate for further typing; although this is not considered a viable option at the local hospital level. As clinical and public health management of individual cases and outbreaks requires confirmation and characterisation of cultured isolates, there is uncertainty over how to action a PCR positive STEC result when culture on CT-SMAC is negative.

The aim of this presentation is to (i) review the current options we have for improving culture of non-O157 STEC today (ii) explore how best to manage the challenges and (iii) look forward to the technological advances that might be available to us tomorrow.

STEC at the Interface: Navigating One Health Challenges through an Indian lens.

Dr Robert Atterbury[1]

[1] University of Nottingham

Shiga toxin-producing E. coli (STEC) is an archetypical One Health pathogen, sitting at the interface between human, animal, and environmental health. Yet strategies for controlling STEC are largely shaped by evidence from high-income countries, which often overlook the complexity of endemic settings elsewhere.

Using Nagpur, Central India as a case study, this talk explores the challenges of controlling STEC where multiple reservoirs, informal food systems and limited surveillance infrastructure intersect. It examines the practical difficulties of detection, attribution and control and considers how India's new National One Health Institute, based in Nagpur, can help address fundamental knowledge gaps and inform how One Health frameworks need to adapt for STEC control outside of high-income contexts.

A reflection on the main challenges for STEC research

Prof David Gally[1]

[1] The Roslin Institute, University of Edinburgh

The talk will aim to summarise key progress in STEC research presented at the meeting and provide my thoughts on areas for further research and development. These will include the need to continue to develop our understanding of the biology of STEC in ruminant hosts and how this relates to the emergence of strains that are a threat to human health. Specifically, do Shiga toxins provide an advantage to the E. coli population that express them in cattle and what significance is the prophage context for Shiga toxin beyond transmission between E. coli hosts. What can STEC genomics reveal about the most dangerous strains so that they can be predicted and potentially indicate what practices may reduce the selection of such pathogenic strains in reservoir hosts. In relation to interventions, I will review the challenge of reducing STEC O157 colonisation and excretion from cattle including a brief summary of our own vaccine and phage-based research.

Oral Presentations

Diversity of clinical isolates of Shiga toxin producing Escherichia coli in Canada

Dr. Kyrylo Bessonov[1], Ms. Julie Shay[2], Ms. Tanis McMahon[3], Dr. Kelly Weedmark[3], Dr. Catherine Carrillo[4], Dr. Alexander Gill[3]

[1] Public Health Agency of Canada, National Microbiology Laboratory,
[2] Health Canada, Bureau of Data, Science and Knowledge Integration,
[3] Health Canada, Bureau of Microbial Hazards,
[4] Canadian Food Inspection Agency, Ottawa Laboratory (Carling)

Genomic characterization of clinical isolates of Shiga toxin producing Escherichia coli (STEC) should guide development of methodologies for diagnostic testing and food safety programs. We report here the first national overview of the genotypes of clinical STEC in Canada. The data for this overview was provided by PulseNet Canada, a national surveillance system for identifying outbreaks of foodborne illnesses, to which public health partners submit the genomes of priority pathogen isolates.

A total of 6,188 E. coli genomes submitted to PulseNet Canada from 2018 to 2024 were determined, with ECTyper v2.0.0, to possess stx and were classified as STEC. The dataset comprised 2,394 genomes linked to 476 outbreaks, and 3,794 genomes from sporadic cases of illness. STEC O157:H7 accounted for 48.3% of 476 outbreaks, and 23.4% of 3,794 sporadic cases. Excluding O157, and genomes which could not be O-typed, 104 O-types were present. Stx-subtypes encoded in the genomes included 1a, 1c, 1d, 2a to 2h, 2j, 2k, 2n, and 2o. Outbreak genomes encoded Stx-subtypes 1a, 1c, 2a to 2d, and 2o. Intimin positive (eae+) STEC accounted for 94.7%(n=451) of outbreaks and 83.3%(n=3,170) of sporadic cases. The most common virulence profile was stx1a, eae+ in outbreaks (32.6%) and sporadic cases(45.9%). Forty-six STEC genomes encoded virulence factors associated with other E. coli pathotypes, enterotoxigenic, enteroinvasive, enteroaggregative, and diffuse adherent.

The observed diversity of Stx-subtypes and serotypes of clinical STEC isolates indicates that methods for diagnosis and food analysis should be adopted that are serotype independent and inclusive of diverse Stx-subtypes.

Estimating the Burden of STEC in Scotland – A Data Linkage Study (2015-2023)

Ms Susan Brownlie[1], Dr Alison Smith Palmer[1], Dr Lesley Allison[2], Dr Anne Holmes[2], Dr Jane Horne[3], Dr Jacqui McElhiney[3]

[1] Public Health Scotland, [2] Scottish E. coli O157/STEC Reference Laboratory, [3] Food Standards Scotland

The incidence of STEC in Scotland has been consistently high compared to other comparable countries, with the burden not evenly distributed across the population of Scotland.

A comprehensive data linkage study was undertaken using all laboratory confirmed cases of STEC in Scotland 2015-2023, totalling over 2500 cases. Laboratory data, including Whole Genome Sequencing information on stx subtype and other key virulence genes, was linked to epidemiological, exposure and symptom data from surveillance questionnaires, hospitalisation data, cancer registry data, community prescribing data, Charlson comorbidity index, deprivation and rurality data. This provided a detailed insight into the burden of infection including factors associated with severe clinical presentation, hospitalisation rates and duration, HUS and longer-term sequelae.

Isolates were coded according to their JEMRA classification. Severity of infection did not always correspond to those stx profiles considered under JEMRA to have the greater or lesser potential for severe infection.

Living in a rural community was associated with a greater risk of becoming infected with STEC and the strain profile. This has implications when considering risk communication of those living in rural communities and healthcare professionals.

The size of the dataset together with the diverse linked data sources provides a comprehensive understanding of the burden of STEC infection in Scotland and an evidence base to help inform future guidance and research.

Population analysis and host-disease associations of STEC from various sources across eleven European countries using whole genome sequencing

Rosangela Tozzoli[1], Dr Tristan Schadron[2], Arnold Knijn[1], Luca De Sabato[1], Stefano Morabito[1], Margherita Montalbano di Filippo[1], Eve Fiskebeck[3], Gro Johannessen[3], Jeevan Karloss Antony-Samy[3], Linnea Good[4], Robert Söderlund[4], Angela van Hoek[2], Lapo Mughini-Gras[2], Eelco Franz[2], Kinga Wieczorek[5], Gaia Scavia[1], Ornella Moro[1], Paola Chiani[1], Valeria Michelacci[1], Catherine Burgess[6], Geraldine Duffy[6], John Rodgers[7], Miranda Kirchner[7], Angela Pista[8], Leonor Silveira[8], Ana Amaro[9], Lurdes Clemente[9], Marie Chattaway[10], Timothy Dallman[11], Susanne Schjørring[12], Flemming Scheutz[12], Brian Byrne[13], Montserrat Gutierrez[13], Vicente Lopez-Chavarrias[14], Maria Ugarte-Ruiz[14], Lin Brandal[15], Umaer Naseer[15], Ivana Kolackova[16], Aldert Zomer[17], Jaap Wagenaar[17], Sara Pires[18], Tine Hald[18], Dr Claire Jenkins[10], Camilla Sekse[3]

[1] Istituto Superiore Di Sanità, Rome, Italy, [2] Centre for Infectious Disease Control, National Institute for Public Health and the Environment (RIVM), [3] Norwegian Veterinary Institute, [4] Swedish Veterinary Agency (SVA), [5] Department of Microbiology of Food and Feed, National Veterinary Research Institute, [6] Teagasc Food Research Centre, Ashtown, [7] Department of Bacteriology, Animal and Plant Health Agency, [8] National Institute of Health Doutor Ricardo Jorge, [9] National Institute of Agrarian and Veterinary Research, [10] Gastrointestinal Bacteria Reference Unit, United Kingdom Health Security Agency, [11] Faculty of Veterinary Medicine, Institute for Risk Assessment Sciences (IRAS), Utrecht University, [12] Department of Bacteria, Parasites and Fungi, Statens Serum Institut, [13] Food Microbiology Division, Department of Agriculture Food and the Marine, Backweston Laboratory Campus, [14] VISAVET Health Surveillance Centre, Universidad Complutense Madrid , [15] Norwegian Institute of Public Health, [16] Department of Public Health, Faculty of Medicine, Masaryk University, [17] Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, [18] National Food Institute, Technical University of Denmark

Background

Shiga toxin-producing Escherichia coli (STEC) are important foodborne pathogens. In the DiSCoVeR project (https://onehealthejp.eu/jrp-discover/) a STEC inventory from human and non-human sources from 11 countries was set up and ≥ 3500 strains were sequenced to perform comparative genomics analysis. We used this dataset to assess STEC population structure and to investigate potential associations between genomic features, host reservoirs and symptoms.

Rationale for the work
Most STEC isolates analysed by WGS in this study were collected between 2010-2020. An ad hoc pipeline was used for serotyping, stx subtyping, 7-loci MLST, virulotyping and cgMLST. The results were analysed with Principal Component Analysis (PCoA) together with isolation source to assess clustering of STEC subpopulations.

Main findings
PCoA revealed three distinct human STEC subpopulations (STEC_1, STEC_2 and STEC_3) grouped by symptoms, which were further analysed for associations between genomic features and variance. For non-human STEC, PCoA showed a more dispersed distribution, except for one subpopulation with genes linked to specific host species, and some virulence profiles overlapping with the STEC_1 population.

Conclusions
Our analysis identified distinct STEC subpopulations from human cases, each characterized by specific genetic features and associated with varying proportions of severe disease outcomes. These findings provide novel insights supporting the risk assessment of STEC.

The long and the short of it: Utilising multiple genome sequencing methods for the characterisation of Shiga Toxin-Producing Escherichia coli.

Dr David Greig[1], Anaïs Painset[1], Dr Marie Chattaway[1], Dr Claire Jenkins[1]

[1] Gastrointestinal Bacteria Reference Unit, UKHSA, [2] NIHR Health Protection Research Unit for Gastrointestinal Pathogens, [3] NIHR Health Protection Research Unit in Genomes and Enabling Data

Background:
Processes that contribute to clinical management of patients and public health surveillance systems are regulated, monitored and controlled by clinical governance frameworks and quality systems. The fast-moving field of long-read genomics it is challenging to standardise a fixed bioinformatics workflow due to the rapid iteration and updates to software. To address this, we introduce Cóimeáil, a nextflow-based bioinformatics pipeline which utilises both assembly dependent and assembly independent analyses to derive STEC typing data in a dual format.

Rationale:
With the advent and development of long-read sequencing technologies, we can now generate highly accurate complete contiguous de novo assemblies of complex STEC genomes. This enhances and expands the exploration, characterisation and typing of elements of the accessory genome of this important zoonotic, foodborne pathogen.

Main findings:
Interrogation of the accessory genome of STEC has enabled us to identify highly pathogenic emerging clones of STEC by monitoring the loss, acquisition and detection of multiple copies of stx-encoding bacteriophages, plasmids and other mobile genetic elements. We have observed large chromosomal rearrangements in persistent STEC strains causing large, recurrent outbreaks that may enhance survival in the animal reservoir and the environment.

Conclusions:
Use of a UKAS accredited routine, long read sequencing pipeline facilitate exploration of the STEC accessory genome, improving our ability to predict emerging threats within the animal reservoir and food chain, provide accurate risk assessments during outbreak investigations and a better understand genome plasticity and the drivers of STEC evolution.

Food safety implications of antimicrobial resistance in leafy vegetables irrigated with reclaimed water

Dra Pilar TRUCHADO[1], Jesús López-Cañizares[1], Alberto Martínez-Alonso[1], Prof Ana Allende[1]

[1] CEBAS-CSIC

The reuse of reclaimed water for agricultural irrigation is supported to enhance sustainable water management and address water scarcity. However, its implementation has prompted public health concerns regarding the potential transfer of antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs) from irrigation water to agricultural crops. This study evaluated the occurrence and abundance of ARB and ARGs in lettuce irrigated with three water sources of contrasting microbiological quality: municipal tap water (control), reclaimed secondary-treated water (lowest quality), and reclaimed tertiary chlorine-treated water (highest quality). Irrigation was applied throughout the entire crop cycle. Plant samples were collected before irrigation started and before harvest, when plants reached commercial maturity, allowing the assessment of changes in plant-associated antimicrobial resistance during cultivation. Culture-based analyses targeting Escherichia coli and ESBL-producing E. coli, together with quantitative PCR and metagenomic sequencing, were applied to characterize plant-associated ARB, ARGs and microbial communities. Higher abundances of ARB and ARGs conferring resistance to β-lactams, macrolides, tetracyclines and aminoglycosides were detected in plants irrigated with secondary-treated water, whereas irrigation with tertiary-treated reclaimed water resulted in substantially lower levels. Differences in the composition and abundance of the plant-associated resistome were observed depending on irrigation water quality. Overall, the results indicate that while leafy vegetables may carry antimicrobial resistance determinants, the use of adequately treated reclaimed water substantially mitigates potential food safety risks. These findings support the safe agricultural reuse of reclaimed water, provided that appropriate water quality standards and irrigation management practices are applied along the production chain.

Food attributable proportion of domestic STEC infections in New Zealand

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Background
New Zealand has one of the highest reported rates of Shiga toxin-producing Escherichia coli (STEC) infections among developed countries, yet most cases are sporadic with unclear sources. Understanding transmission pathways is essential for effective food safety interventions. Previously, expert estimates suggested 20–40% of STEC infections may be foodborne, but supporting data were limited.

Study Rationale
This study aimed to refine attribution of STEC infections to transmission pathways and assess whether epidemiological and genomic data could identify foodborne sources. The goal was to inform future strategic direction for New Zealand Food Safety in this area and enhance public health protection.

Main Findings
Analysis of 6,258 notified cases (2016–2023), hospital discharge data, and whole genome sequencing (WGS) of 3,221 clinical isolates revealed direct attribution to foodborne transmission was not possible due to data limitations. Using an exclusion-based approach, an estimated 10–14% of cases were travel-related, 2–9% were person-to-person transmission, and 53–66% were linked to rural exposures. The 22–36% of domestically acquired cases were attributed to 'other' pathways, including foodborne transmission. Genomic cluster analysis suggested possible links to widely distributed foods, though specific sources could not be identified. Raw milk remains the only food with confirmed epidemiological and genomic linkage to STEC cases.

Conclusions
Foodborne transmission remains a plausible contributor to domestically acquired STEC infections in New Zealand, but current surveillance and data systems limit precise attribution. Enhanced food exposure data, expanded WGS of STEC isolates, and targeted microbiological surveys would improve source attribution and help guide effective food safety policy.

Wildlife crop damage as a potential source of foodborne STEC infection

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Background
Wildlife is a known but underexplored source of human STEC infection, with contaminated agricultural produce and game meat representing likely routes of transmission. Current knowledge gaps include the prevalence, molecular epidemiology and phenotypic traits of wildlife STEC as well as stakeholder risk perception.

Rationale for work
A better understanding of wildlife STEC will benefit a safe and sustainable food production and help minimize human-wildlife conflict. In a four-year project 2024-2027 we are investigating wildlife associated with crop damage as sources of STEC in Sweden. We have sampled wild boar, deer and large grazing birds, as well as field-grown produce at the retail level. Recovered isolates have been characterised and compared to each other and STEC from livestock and humans.

Main findings
Wild boar were found to have a high prevalence of STEC with stx2e (e.g. O100:H30, O8:H19, ONT:H21), likely posing limited risk for consumers. Both deer and large grazing birds in contrast frequently carried strains with stx2b and stx2g, some of which are also comparatively common among humans in Sweden (e.g. O187:H28, O146:H28, O54:H45) but rarely or never associated with large outbreaks or HUS. All wildlife STEC strains were intimin negative and hybrid pathotypes common. All food samples analysed in the project have been negative.

Future directions
In the next phase of the project, views and practices regarding transmission of foodborne infection from wildlife will be explored among farmers and responsible authorities. Recovered wildlife strains will be further characterized to reveal evidence of host or niche adaptation.

DERIVE - Detection and Risk management of VTEC in groundwater

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Background
Unregulated private groundwater supplies are widely used for drinking water in Ireland, representing an important VTEC transmission route. The DERIVE project (2022-2026) aims to design and validate tools for detection and risk management of VTEC in groundwater.

Methods
To detect VTEC serogroups, a low-volume filtration/lysis method was developed and combined with quantitative real-time PCR of O157 and O26 serogroup-specific genes. Groundwater wells (n=12-14 per catchment) were sampled fortnightly for one year in 2 distinct hydrological catchments to quantify O157/O26 (n=592 samples). Three other catchments were chosen and sampled fortnightly over 16 summer/autumn weeks (n=234 samples) to validate a multi-criterion catchment contamination risk-mapping tool. E. coli positive samples (Colilert-18) were filtered, enriched, and stx1/2 PCR was employed for VTEC detection. Metadata on climate, hydrogeology, land use, and well infrastructure was analysed to investigate sources and transmission pathways.

Main Findings
VTEC sample positivity ranged from 0-63% between catchments. Detection rates broadly corresponded to groundwater vulnerability classifications and seasonal infection peaks.

Logistic regression identified contrasting predictors for O157 and O26 presence in a karstic catchment, with cattle density (OR 1.001, p=0.001) and monthly rainfall (OR=1.010, p=0.019) associated with O157, whereas septic tanks (OR=1.022, p=0.042) and drought (OR = 0.990, p = 0.023) predicted O26.

During summer/autumn 2025, VTEC (stx1/stx2) was detected in 28/234 (12%) samples, representing 41% of E. coli positive samples. Serogroups O157 (29% VTEC samples), O145 (29%), O104 (25%) and O103 (21%) were detected.

Conclusion
The tools developed may prove useful in targeted risk management of groundwater wells for VTEC.

Multitarget Early Management of Bloody Diarrhea and Hemolytic Uremic Syndrome: STEC Point-of-Care Testing, Volume Expansion, and Azithromycin Use

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Background
Early identification of Shiga toxin–producing Escherichia coli (STEC) is critical to prevent hemolytic uremic syndrome (HUS).

Rationale
We implemented a standardized multitarget early management protocol for pediatric patients with bloody diarrhea, integrating rapid STEC point-of-care testing, microbiological confirmation, isotonic volume expansion, and azithromycin therapy.

Main findings
Between January 1 and December 15, 2025, all children aged 6 months to 14 years presenting with acute bloody diarrhea were managed according to a predefined institutional protocol. Initial evaluation included laboratory assessment, a rapid immunochromatographic IgM anti-STEC test (CHEMSTRIP® E. coli IgM O157/O145 Card Test; Chemtest S.A., Argentina), and stool culture with PCR confirmation.

We conducted a retrospective analysis of anonymized clinical data obtained during routine clinical care.

Seventy-six (76) determinations were performed. STEC infection was confirmed in 2 cases (2.6%). The rapid STEC test showed no false-negative results and three false-positive results. Using stool culture and PCR as the reference standard, sensitivity was 100%, specificity 95.9%, negative predictive value 100%, and positive predictive value 40%. False-positive results were mainly associated with co-infections and biological cross-reactivity.

Rapid STEC testing enabled early risk stratification and supported timely clinical decisions, including safe discharge of low-risk patients. Azithromycin did not prevent HUS development but improved symptoms, was not associated with disease worsening or adverse events, and resulted in negative follow-up stool cultures.

Conclusions
In a low-prevalence setting, rapid STEC point-of-care testing demonstrated excellent negative predictive value and clinical utility. A multitarget strategy may optimize clinical management and epidemiological control in children with bloody diarrhea.

Detection and characterisation of Non-O157 Shiga Toxin-Producing Escherichia coli on Scottish Deer Farms

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Shiga-toxin producing Escherichia coli (STEC) are a group of zoonotic enteric pathogens that cause foodborne human infections globally. In the UK, STEC serogroup O157 (STEC O157) strains are responsible for most clinical cases, however the incidence of non-O157 STEC clinical cases has increased in recent years. Ruminant livestock are considered the primary reservoir of STEC, and deer are now considered an important species in the STEC transmission cycle. We previously estimated the prevalence of STEC O157 and non-O157 STEC in wild Scottish deer as 0.28% and 69.5%, respectively. In contrast, STEC O157 was detected in 9.65% of Scottish farmed deer, closer to the levels present in Scottish cattle.

We aimed to detect and characterize non-O157 STEC strains present on Scottish deer farms. Faecal samples were collected on 10 Scottish deer farms, four of which were mixed enterprises that also farmed cattle and/or sheep. Samples were enriched, plated on SMAC agar and colonies screened for the presence of stx1, stx2 and eae genes. Whole genome sequencing was performed on two positive isolates per species from each farm. Additional isolates were also collected from mixed enterprises to investigate the impact of cattle and/or sheep on non-O157 STEC diversity.

To date, >80 non-O157 STEC strains have been identified across 10 deer farms. Initial sequencing of 30 strains has identified 16 different O-types. Further sequencing is underway to determine whether strains are host-species specific, and to evaluating public health risk by assessing genetic relatedness to strains associated with human clinical disease.

The antibiotic NAB815: a new tool in the prevention of typical hemolytic uremic syndrome

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Background

Typical HUS is in most cases associated with infections by Escherichia coli strains producing Stx2. The microangiopathic injuries related to toxin action occur mainly in the microvasculature of the kidney when the specific glycolipid Gb3Cer is targeted by the toxin. In human blood Stx2 is intercepted by a naturally occurring blocking factor (HuSAP) which prevents its toxic action. For this reason, Stx2 does not enter the kidney directly but via a tortuous path, i.e. it binds to circulating cells and is subsequently delivered in extracellular vesicles. The crucial binding of Stx2 to human circulating cells occurs through Gb3Cer and TLR4.

Rationale for the work

Interfere with Stx2/TLR4 interactions to prevent the release of pathogenic extracellular vesicles responsible for HUS development.

Main findings or outcomes

We have found a specific inhibitor of Stx2/TLR4 interactions, a preclinical polymyxin B derivative called NAB815. The compound binds to Stx2 with high affinity and impairs the formation of Stx2-containing pathogenic extracellular vesicles produced by human leukocytes and platelets. NAB815 significantly decreases the toxic cargo

of the vesicles and reduces their toxic effects for Vero cells. CD-1 mice intoxicated with human-derived Stx2-containing extracellular vesicles or free Stx2 showed reduced renal impairment in the presence of sub-bactericidal (0.01 µg/ml) NAB815 concentrations.

Conclusions
NAB815 is significantly less nephrotoxic than polymyxin B and is effective at sub-bactericidal concentrations, thus overcoming the concern that antibiotics are harmful to patients with STEC infections. Administration of NAB815 would represent a useful approach in preventing or mitigating HUS.

Azithromycin for the prevention of Hemolytic Uremic Syndrome in Shiga toxin-related diarrhea. Data from the ItalKid-HUS Network.

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Background:
Early diagnosis of Shiga toxin–producing Escherichia coli (STEC) infection before the onset of hemolytic uremic syndrome (HUS) has become increasingly common, offering a valuable therapeutic window currently limited to supportive care. Antibiotic use is traditionally discouraged due to concerns that it might trigger STEC-HUS, a notion largely based on evidence involving bactericidal agents. Several in vitro and in vivo studies both on animal models and humans indicate that azithromycin may be safe and prevent STEC-HUS or reduce its severity.

Methods:
Within the ItalKid-HUS network devoted to the screening of children with acute bloody diarrhea (ABD) for Shiga toxin (Stx) genes, since January 2024 all Stx-positive patients have been treated with oral azithromycin 10 mg/kg/day until diarrhea resolution (maximum 5 days).

This analysis evaluates whether azithromycin reduces progression to HUS or mitigates its severity in STEC-infected patients, using as control group Stx-positive patients identified from January 2014 to December 2023.

Results:
Ninety-five patients (45 females, median age 5.8 years) with Stx-positive diarrhea received azithromycin. Treatment began a median of 4 days after symptom onset (3 days after ABD onset) and lasted a median of 5 days. Among treated patients, 13

were Stx1-positive, 40 were Stx2-positive, 27 were positive for both toxins, and 15 for unspecified Stx type. HUS developed in 5/95 treated patients (5.3%) versus 13.2% in the control group. No treatment-related side effects have been observed.

Conclusions:
Azithromycin is safe and may represent a useful therapeutic option in Stx-positive patients to prevent HUS or to mitigate its severity.

Genomic Insights into Within-Farm Persistence and Global Phylogenetic Relatedness of STEC O26 in a Dairy Cattle Farm in the UK

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Background:
Historically, the dominant Shiga toxin-producing Escherichia coli (STEC) serotype associated with human infections globally was O157. However, over the last decade reports of non-O157 STEC cases have increased, with STEC O26 in particular rising and associated with severe disease such as haemolytic uraemic syndrome. Cattle are an important STEC reservoir and often linked epidemiologically to human outbreaks involving contaminated foods.

Rationale:
A year-long pilot study on a cattle dairy farm in England was undertaken to investigate the occurrence, persistence, and genomics of STEC O26. A total of 250 faecal and environmental samples were collected from young and adult cattle over four visits.

Main findings:
A total of 23 E. coli O26 isolates were recovered. Whole genome sequencing showed twelve isolates were sequence type (ST) 21 carrying stx1a, stx2a, and eae, and eight isolates were ST29 carrying eae; all belonged within multi-locus sequence type clonal complex 29. Genomic analysis found a clonal population of STEC O26 circulating on farm, while other isolates were very closely related. Positive STEC O26 samples were significantly associated with the third visit compared to the fourth visit (Chi2, $p = <0.05$). Bayesian logistic regression modelling showed temporal and environmental variation in STEC detection, with reduced odds for detection outdoors.

Conclusions:
Despite low detection rates, STEC O26 persisted on a dairy cattle farm in England over a year; reinforcing public health risks associated with cattle as STEC reservoirs. Therefore, high maintenance of hygiene standards and protocols remains necessary to protect the public from STEC infection.

Whole-genome analysis of antimicrobial resistance genes and mutations in STEC isolates from Danish patients, 2020–2025

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Shiga toxin-producing Escherichia coli (STEC) infections are usually self-limiting, but antimicrobial treatment can be necessary in some cases. However, some antimicrobials may increase Shiga toxin production, raising concern about their use. We investigated the occurrence of genetic antimicrobial resistance (AMR) markers in Danish STEC isolates using a whole-genome sequencing (WGS)-based approach. A total of 1607 STEC isolates from Danish patients isolated between 2020 and 2025 were analysed using Illumina paired-end sequencing, SKESA assembly, and the AMRFinderPlus (NCBI) database to identify AMR genes and point mutations (PMs). Serotypes and stx subtypes were assigned using an in-house workflow.

The most frequent serotypes were O157:H7 (13%), O103:H2 (10%), O63:H6 (6%), O26:H11 (6%), and O146:H28 (5%). Haemolytic uremic syndrome (HUS)-associated stx genes, stx2a and/or stx2d, were identified in 10% of isolates. The AMR marker prevalence was 21% for aminoglycosides, 6% for β-lactams, 1% for macrolides, 4% for phenicols, 9% for quinolones, 11% for sulfonamides, 8% for tetracyclines, and 7% for trimethoprims. Four isolates carried aac(3)-IId (gentamicin resistance), two carried blaCTX-M-15 (cephalosporin resistance), and 76 carried blaTEM-1 (ampicillin resistance). Macrolide resistance genes included mph(A) (9 isolates), mph(B) (8) and mph(G) (2). Frequent quinolone-associated PMs were parE_I355T (79), and gyrA_S83L (34). Plasmid-encoded qnr genes were detected in 8 isolates.

Overall resistance to commonly used antimicrobials such as azithromycin and third-generation cephalosporins were low. The observed AMR markers suggests that treatment strategies avoiding fluoroquinolones and β-lactams are feasible, thereby limiting the use of agents known to induce the bacterial SOS response and potentially enhance Shiga toxin production.

Gut STEC infection impairs the central pacemaker Suprachiasmatic nucleus gene expression through the gut-brain axis

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Background:
Shiga toxin-producing Escherichia coli (STEC) is a foodborne pathogen that causes not only diarrhea but bloody diarrhea, hemolytic uremic syndrome (HUS), and encephalopathy in severe cases. Shiga toxins (Stxs) play a central role in pathogenesis. Recently, we reported that Stx2 treatment induces differential expression of certain circadian rhythm genes in mouse kidney and human renal proximal tubular cells.

Rationale for the work:
Stx exerts its toxicity on cells such as renal cells and neurons through the receptor Gb3. It has also been demonstrated that the Gram-negative outer membrane factor lipopolysaccharide disrupts the central pacemaker, the suprachiasmatic nuclei (SCN), in rodents. Therefore, we hypothesized that circadian rhythm genes are impaired in the SCN after oral STEC infection using B2F1 (O91:H21) in a mouse model.

Main findings:
The number of oscillating genes was 381 in PBS, 727 in both PBS and STEC strain B2F1, and 4196 in B2F1 groups. This result suggests that more than 4,000 genes begin to oscillate after B2F1 infection. Differential oscillation patterns of circadian genes between PBS and B2F1 groups included Clock, Per1, Per3, Ccr4nl, and Nfil3, suggesting involvement of the circadian gene regulatory regions E-box and D-box.

Conclusions:
The data suggests that enteric STEC infection influences SCN by altering gene expression in circadian rhythm, highlighting the novel pathological significance of enteric infection on brain dysfunction. The circadian genes may be targeted as therapeutic markers for STEC infectious disease, for which currently no specific therapy is established.

Long-term outcomes following Shiga toxin-producing Escherichia coli (STEC) infection: A prospective longitudinal outbreak cohort study

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Background
In September 2023, Calgary, Alberta, Canada, experienced a point-source daycare E. coli O157 outbreak resulting in 273 laboratory-confirmed cases among facility attendees. The spectrum of severity in this circumscribed cohort spanned from asymptomatic infection to anuric hemolytic uremic syndrome. Recognizing the absence of robust long-term outcome data among STEC-infected children, we leveraged this outbreak to conduct a follow-up study evaluating intermediate-term clinical and laboratory outcomes.

Methods
We conducted a prospective longitudinal cohort study aiming to recruit all exposed children - infected cases and uninfected controls. Eligible children were aged 9 months to <6 years at the time of exposure, attended an affected daycare and were tested for STEC infection. Our objective was to determine if children infected with E. coli O157 experience greater sequelae in pre-defined domains than those who were uninfected, and to evaluate if the magnitude of deficit is proportional to acute infection severity. We assessed outcomes in four domains at 6-, 12- and 24-months post-infection/exposure: 1) general/inflammatory/hemolysis; 2) kidney; 3) gastrointestinal; and 4) cardiometabolic.

Results
Data analysis is in progress and will be completed by January 2026.

Conclusion
Pending.

EHEC O45:H2 Outbreak in Germany in 2025

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In autumn 2025, an outbreak of shigatoxigenic Escherichia coli (STEC) including cases of haemolytic uraemic syndrome (HUS) occurred in Germany. At onset of the outbreak, the majority of cases were concentrated in northeastern Germany, later shifting to the south-west. According to the case definition, more than 400 cases were associated with the outbreak, of which ~200 were confirmed by molecular methods. A total of 63 patients -mainly children- developed HUS and three individuals died.

The outbreak strain was identified as STEC O45:H2, ST301, stx2a and eae-xi positive and exhibits the following antibiotic resistances: ampicillin, chloramphenicol, kanamycin, nalidixic acid, trimethoprim/sulfmethoxazole, trimethoprim and tetracycline. More than 140 isolates could be assigned to the outbreak cluster by genome analysis using cgMLST. Pairwise allele distances ranging from 0 to 10 and above within the cluster indicate an unusual mutation rate. Long read sequencing revealed four plasmids, one of them carrying resistance genes. Further genetic, molecular and biological analyses are ongoing to determine the virulence factors and characteristic of the outbreak strain.

We present another example where STEC of a rare serovar, causes a large outbreak in Germany. Prior to the outbreak, 2015 to 2025, the German Reference Center for Salmonella and Other Bacterial Enteropathogens detected only 13x O45:H2 strains among over 10 000 analysed samples in the genome-based national surveillance of clinical STEC four associated with HUS. This demonstrates, the relevance of rare STEC serovars and the power of integrated genomic surveillance to gain an overview on national and global levels.

Like finding a needle in a haystack? The difficulties of isolating STEC from implicated food vehicles in an outbreak investigation

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Investigations into foodborne disease outbreaks use epidemiological, microbiological and food chain information to identify the contaminated food vehicle and inform risk management actions.

In 2024 we published a systematic review looking at how often the outbreak strain was isolated from implicated food or environmental samples during an STEC outbreak investigation, and the factors that contributed to the success or otherwise of isolation.

Our original review covered the period 2000 - 2019, and in this paper we used the same search strategy to identify publications from 2020 - 2025 to update and re-explore our original conclusions that lack of sample availability and methodological issues were the principal reasons why the outbreak strain was not successfully isolated. The update period will broadly reflect the global introduction of whole genome sequencing, and we will consider how that has affected the identification of outbreaks and the impact on the success of isolation of the outbreak strain from food or environmental samples.

Shiga toxin breaks the epithelial barrier and induces inflammation in a human 3D gut-on-a-chip model

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Background
Shiga toxin (Stx), the cardinal virulence factor of Stx-producing E. coli (STEC), is a potent AB5 toxin that irreversibly inhibits eukaryotic protein synthesis thus leading to host cell death. There are two immunologically distinct Stx types, Stx1 and Stx2, that are further divided into subtypes, with Stx2a being the one most often associated with severe clinical course. Animal models have been crucial for studying STEC-host interactions; however, none of the currently available ones fully reflects STEC pathogenesis in humans.

Rationale for the work
We evaluated the effects of bacterial culture supernatants and purified Stx on the epithelial barrier, inflammation and cell viability in a human 3D gut-on-a-chip model.

Main findings / outcomes
Bacterial culture supernatants of Stx2a-producing E. coli O104:H4 but not E. coli O104:H4 Δstx caused disruption of the epithelial barrier and increased IL-8 secretion in the 3D gut-on-a-chip model after 48 h of treatment, suggesting that Stx2a is the virulence factor responsible for these phenotypes. At this point, no loss in the epithelial cell viability associated with the presence of Stx2a was detected. Experiments with purified toxin confirmed that both Stx1a and Stx2a alone could cause the effects on epithelia barrier integrity and inflammation. The virulence potential of bacterial supernatants from clinical STEC isolates expressing different Stx1 and Stx2 subtypes was tested using the model.

Conclusions
This human 3D gut-on-a-chip model serves as a suitable system to study STEC virulence and provides valuable insights into STEC course of infection.

Genotoxins in the Gut: A New Key to Understanding Stx Phage Induction and HUS ?

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Hemolytic uremic syndrome (HUS) is most frequently caused by infection with Shiga toxin-producing Escherichia coli. The strains most strongly associated with the development of HUS produce Stx2, with Stx2a and Stx2d being the variants most often linked to severe disease. These toxins are encoded by temperate bacteriophages whose induction controls toxin expression. During the lysogenic state, the stx genes remain silent, but DNA damage activates the bacterial SOS response, initiates the lytic cycle, and leads to high level toxin production that drives the onset of HUS. Determining which factors promote the induction of Stx2 phages is therefore essential for understanding the disease progression. We hypothesized that the gut microbiota could create microenvironments favorable to prophage activation. Colibactin, a genotoxic metabolite, is produced by E. coli strains found in the gut microbiota of approximately one in five individuals. We demonstrated that colibactin induces the lytic cycle of Stx2 encoding phages, providing direct evidence that microbiota derived genotoxins can activate phages carrying the clinically important Stx2a and Stx2d toxins. To study these interactions in vivo, we developed a vertical mother offspring transmission mouse model in which mice are colonized at birth with genotoxic E. coli and infected at adulthood with E. coli producing Stx2a or Stx2d. This model offers a physiologically relevant system to examine how genotoxic strains influence Stx2 phage induction within the gut environment. Overall, our findings identify colibactin producing bacteria as potent inducers of Stx2 phages and reveal a microbiota driven mechanism that may increase individual susceptibility to HUS.

Improving chewBBACA Enhances cgMLST Clustering Consistency in STEC Surveillance

Dr Sofie Holtsmark Nielsen[1], Dr Rasmus Amund Henriksen[1], Dr Kasper Rømer Villumsen[1], Mrs Gitte Sørensen[1], Dr Katrine Grimstrup Joensen[1], Dr Susanne Schjørring[1], Dr Kristoffer Kiil[1]

[1] Statens Serum Institut

After the discontinuation of Bionumerics (BN), many public health laboratories have transitioned to open-source solutions for core-genome multilocus sequence typing (cgMLST) across bacterial pathogens. Especially, chewBBACA, which is adopted by both ECDC and EFSA, is now increasingly applied in STEC surveillance. However, during implementation in our genomic surveillance, we observed increased intra-cluster allelic distances (AD) compared with BN, raising concerns about the consistency of cluster definitions, which rely on narrow, stable allelic-distance thresholds for outbreak detection.

We analysed 532 isolates encompassing all Danish STEC surveillance isolates from 2024, as well as major historical clusters within ST11, ST17, ST21, ST32, and ST583. The mean intra-cluster distance increased from 3 AD with BN to 5.3 AD with chewBBACA, accompanied by a decline in median call percentage from 99.1% to 95.8%. Investigation revealed that pyrodigal-based gene finding in chewBBACA was the main contributor, as it occasionally failed to identify the correct open reading frames.

To address this, we introduced a blastn-based gene-calling step targeting schema alleles directly in the assemblies, performed schema curation, and implemented minor corrections to the chewBBACA code. Collectively, these modifications reduced the intra-cluster distance to 3.6, and improved the median call percentage to 98.8%. Although only 769 out of 1.2 million allele calls were divergent (multiple variants called in chewBBACA vs. one variant in BN), they impacted outbreak detection. Their impact highlights the importance of precise allele calling. With these enhancements, chewBBACA can serve as a replacement for BN while maintaining consistent clustering performance in STEC genomic surveillance.

ΦO104 of E. coli O104: a “guided weapon” for rapid colonisation?

Dr Michael Berger[1], Dr Petya Berger, Prof Gerald Koudelka, Prof Ulrich Dobrindt

[1] University Clinic Münster

Background
The exceptional virulence of the German E. coli O104:H4 outbreak strain was attributed to its “hybrid” virulence gene content, which included aggregative adherence fimbriae typical of enteroaggregative E. coli as well as a Shiga toxin 2 (Stx2)–encoding phage, a defining feature of EHEC.

Rationale
Mixed cultures of EHEC and Stx phage-susceptible E. coli were shown to produce higher amounts of Stx than pure EHEC cultures, indicating a role of the microbiota in overall toxin production. However, little is known about the role of the patient microbiome on the course of the disease.

Outcome
As opposed to E. coli O104:H4, the lysogenic state of ΦO104 is unstable in E. coli MG1655, which can lead to an eradication of the strain when co-cultivated with E. coli O104:H4. The stability of the phage in E. coli MG1655 is sensitive to environmental factors, e.g. growth temperature, and to genetic factors, e.g. lsr, indicating that differential stability of ΦO104 is a regulated process. We show that a coordinated SOS response in the absence of an external inducer - that is completely absent in E. coli O104:H4 - precedes the ΦO104 dependent lysis of E. coli MG1655 and that all environmental factors that are stabilizing the phage are as well reducing the extent of the SOS response in the lysogens.

Conclusions
We discuss the potential mechanism resulting in phage auto-activation, therapeutic implications of this aspect of the disease and the role of the specific type of phage in the outcome of the disease.

Use of nanoparticles to improve EHEC confirmation: A new Era for culture media

Dr. Thomas Junillon[1], Benoît Mallen[1], Hugo Beuzelin[1], Lisa Sciandra[1], David Tomas[1]

[1] Biomérieux

Isolation of typical enterohemorrhagic Escherichia coli (EHEC) strains carrying stx and eae genes in food remains one of the most challenging analyses in foodborne pathogen monitoring. Current culture-based approaches require screening numerous colonies—up to 50 according to ISO TS 13136—to verify co-localization of all EHEC determinants, making the process laborious and potentially generating false negatives.

A new culture media (IDEA STEC) has been developed combining conventional culture media, with immunological technologies integrated with functionalized noble metal nanoparticles. Upon specific binding and aggregation in presence of Shiga Toxin production, these nanoparticles induce a measurable shift in light reflection, manifesting as a distinct halo surrounding positive microbial colonies. Nanoparticles from gold and silver at different concentrations (10^{10} to 10^{12} nanoparticles/mL) were tested with different sizes (20 nm to 40 nm) conjugated with specific antibodies against Shiga Toxin. Different antibiotics (Ciprofloxacin, Mitomycin C, Polymyxin B…) at several concentrations were tested in the agar to induce bacterial stress and Shiga toxin production.

Results from the optimized formulation allowed the detection of 360 out of 369 strains of typical EHEC tested. When considering Top 8 STEC (with O80), 98% the strains tested showed typical characteristics.

Overall, this pioneering use of nanoparticle-enhanced culture media opens new avenues for addressing unmet analytical needs in microbiological diagnostics. Its application to complex foodborne pathogen detection as EHEC method, demonstrates significant improvements in confirmation workflows.

Spontaneous induction and characterization of stx-bacteriophages from Shiga toxin-producing Escherichia coli isolates of human and cattle origin

Musafiri Karama[1], Alaba Olawole[1], Mogaugedi Malahlela[1]

[1] University Of Pretoria

Background and Rationale
Bacteriophage-encoded Shiga toxins are the key virulence factor of Shiga toxin-producing E. coli. Stx-bacteriophages are integrated as prophages in the Escherichia coli chromosome and released during the lytic cycle either spontaneously or under the influence of chemical or physical stimuli, thereby influencing STEC evolution and disease outcomes.

Methodology
This study investigated spontaneous stx-bacteriophages induction in 65 STEC isolates. Induced stx-bacteriophages were characterised by PCR for bacteriophages-associated genes including stx subtypes, restriction fragment length polymorphisms and morphology using electron microscopy.

Main findings
Spontaneous bacteriophage induction was observed in 33.8% (22/65) of STEC isolates which released a total of 34 stx-bacteriophages including 13 which carried stx1 and 20 which were stx2-positive. Among the 13 stx1-positive bacteriophages, 69.2% (9/13) possessed both stx1a and stx1c and 4/13 had stx1c only. The 20 stx2-positive bacteriophages consisted of 11 which were concurrently stx2a and stx2c positive and 9/20 which had stx2c only. RFLP analysis of the 12 bacteriophages DNA revealed 6 distinct profiles. Morphological analysis of 21 bacteriophages revealed different bacteriophages shapes: virions with an icosahedral head and a short tail, elongated heads with a long tail, icosahedral head with short and thick or long tail, spherical head and spade-shaped head with a long tail.

Conclusion
Genetically and morphologically diverse bacteriophages which were mostly stx2 positive were induced spontaneously and released from cattle and human STEC isolates. The moderate fraction of spontaneously released stx-bacteriophages which are mostly stx2-positive indicate that stx-bacteriophages may be contributing to STEC evolution, virulence and human disease outcome.

The Rise of Seropathotype type O26:H11 stx2a as a Shiga toxin Producing E. coli Type of Concern in New Zealand

Jackie Wright[2], Dr. Ernest Williams[1], Shevaun Paine[1], Dr. Jing Wang[1], Dr. David Winter[1]

[1] Institute for Public Health and Forensic Science, [2] Institute for Public Health and Forensic Science

Historically in Aotearoa New Zealand (NZ), the primary serotype of concern for Shiga toxin producing E. coli (STEC) infections has been O157:H7 based on its higher likelihood of hospitalisation and progression to severe disease. This serotype is also readily recognisable on selective chromogenic media, making it far and away the most common serotype of STEC identified prior to 2015 when culture independent testing was first implemented in diagnostic laboratories in NZ. Since then, surveillance of all STEC serotypes has become increasingly feasible, including O26:H11, the second most common serotype identified from patient isolates year on year since 2015. Similar to O157:H7, O26:H11 has a high incidence of the 2a sub-type of the Shiga toxin synthesising gene, stx, the most common stx sub-type identified in haemolytic uremic syndrome cases with STEC infections, making O26:H11 stx2a a high-risk seropathotype for personal and public health. Both serotypes demonstrate strong seasonal incidence, although O26:H11 incidence has been steadily increasing in frequency in recent years during Summer months typically dominated by O157:H7, resulting in O26:H11 surpassing O157:H7 as the most common causative agent in haemolytic uremic syndrome cases for 2024 and accounting for 60% of cases in 2025.Risk factors and the demographic groups most affected by this shift in seropathotype will be summarised as well as the difficulty in obtaining comparable surveillance data amongst widespread technological changes in typing.

Advancing phage- based interventions for cattle colonised with E. coli O157:H7

Dr Alison Low[1], Asim Ullah[1], Joel Maki[2], Crystal L. Loving[2], David Gally[1], Jim Bono[3]

[1] Roslin Institute, University of Edinburgh, [2] USDA-ARS National Animal Disease Center, [3] USDA-ARS US Meat Animal Research Center

Shiga toxin–producing Escherichia coli (STEC) O157:H7 remains a public-health and food-safety concern in beef production systems, where pre-harvest shedding contributes to carcass and environmental contamination. Bacteriophages offer a promising targeted intervention. This work aimed to develop an effective phage cocktail to reduce or eliminate STEC O157:H7 colonisation in cattle by identifying phage active under host-relevant conditions and evaluating performance across in vitro, ex vivo, and in vivo models.

Multiple phage demonstrated strong activity in vitro, including in bovine rectal mucus and on epithelial cell–attached bacteria, with several achieving 100-fold reductions. Ex vivo evaluation using faecal samples from colonised cattle confirmed activity of individual phage and cocktails, supporting selection of a five-phage combination for in vivo testing.

Despite activity in vitro and ex vivo, the phage cocktail did not reduce STEC O157:H7 faecal shedding during a controlled cattle challenge in which phage were applied to the terminal colon and rectum. Treatment and control groups showed parallel declines in bacterial shedding indicating failure of effective phage delivery to the rectal colonisation site. The protective mucus layer and probable expulsion of rectally applied liquid phage likely prevented sufficient phage–bacteria contact.

These findings emphasise that while phage selection and cocktail design were effective, delivery to colonising bacteria remains a critical barrier for in vivo intervention. Future work will focus on improved delivery strategies to enable phage access and persistence at the terminal rectum, as well as refinement of the cattle colonisation model to provide a more stable window for intervention assessment.

Mobilome features driving biofilm formation, antimicrobial resistance, and virulence in E. coli O157:H7

Rodolfo Camacho-Martinez[1], Daniel Daniel Mayboca-Padilla[1], Dr Claudia Narvaez-Bravo[1]

[1] University of Manitoba

Background:
STEC remains a major food safety concern because it can persist on food-contact surfaces, withstand sanitation, and carry key virulence and antimicrobial resistance (AMR) genes.

Rationale:
STEC strains vary in their biofilm-forming ability and sanitizer tolerance. This study examined genomic factors underlying differences in O157:H7 survival by comparing 27 isolates from human, cattle, and beef sources.

Main findings:
Beef-derived isolates carried 19 out of 23 key virulence genes (82.6%), followed by human (18; 78.3%) and cattle (17; 73.9%) isolates. Nineteen strains (70.4%) carried at least one of 20 identified AMR genes. Four strains (14.8%) showed qacEdelta1 and qacE, linked to resistance to Quats, while genes like mph(A), sul1, aadA2, and dfrA12 were primarily found in cattle isolates. A total of 48 plasmids were identified, many encoding virulence factors such as ehxA, espP, katP, and toxB. All strains carried core biofilm-related genes. The presence of intact prophage regions suggests phage-mediated transfer contributes to the genetic diversity and virulence potential of these STEC strains. Biofilm phenotypes included 19 non-formers (70.4%), seven weak producers (25.9%), and one strong producer (3.7%). Genes hlya, tccP, stx2, astA, sul1, qacEdelta1, as well as phages and integrons, were positively associated with biofilm formation (Odds ratio > 1).

Conclusions:
O157:H7 isolates exhibited differences in virulence profiles, AMR genes, and biofilm formation. Although most strains were weak or non–biofilm producers, the presence of specific virulence factors, AMR genes, and mobile genetic elements was associated with increased biofilm formation, highlighting their potential role in persistence and resilience to sanitizers.

Enhancing Shiga Toxin–Producing Escherichia coli (STEC) Surveillance Through PulseNet 2.0: Early Detection, Improved Resolution, and Advanced Outbreak Response

Molly Leeper[1], Dr. Jessica Chen[1], Morgan Schroeder[1], Jordan Putney[1,2], Kelley Hise[1], Dr. Heather Carleton[1]

[1] Division of Foodborne, Waterborne, and Environmental Diseases, Centers For Disease Control and Prevention, [2] Applied Science Research and Technology (ASRT), Inc.

Background:
STEC remains an important cause of foodborne and zoonotic illness in the United States. In September 2024, PulseNet USA transitioned to a new cloud-based, modular, open-source platform referred to as PulseNet 2.0. Here, we provide an update on STEC surveillance following this transition, including trends in sequence submissions and outbreak data.

Methods:
The PulseNet 2.0 national Escherichia database was queried for STEC sequence data submitted during October 1, 2024 - December 1, 2025. O-groups, toxin profiles, and sequence types (ST) were evaluated. The System for Enteric Disease Response, Investigation, and Coordination (SEDRIC) was queried for outbreak and source data for the same period.

Results:
9,097 STEC sequences were submitted during the study period. O157 was the most common O-group (21%) followed by O103 (16%), O26 (13%), and O111 (9%). There were 330 unique STs; the top three were ST17 (22%), ST11 (20%), and ST21 (16%). The top three toxin profiles were stx1a (55%), stx2a/stx2c (12%), and stx2a (11%). 566 isolates belonged to 41 unique outbreaks; source information was available for 11 outbreaks. 410 isolates belonged to REPEXH01, a reoccurring STEC O157 strain linked to multiple sources.

Conclusion:
PulseNet 2.0 can be used to detect STEC outbreaks caused by different serotypes and sources and can distinguish them from other E. coli (including Shigella spp.). PulseNet 2.0 was developed to reduce analysis time and increase timeliness of outbreak detection; continued optimization of the STEC workflow will further advance STEC surveillance in the United States.

Cattle dietary component and composition impacts fecal E. coli shedding in Angus Cattle

Mr. Hunter Perez[1], Dr. Andrea Osorio-Doblado[1], Dr. Jeferson Lourenco[1], Dr. Francis Fluharty[1], Todd Callaway[1]

[1] Dept. Animal and Dairy Science, University of Georgia

Cattle fed high starch diets have greater populations of E. coli in the rumen and feces. Abruptly shifting cattle from a high starch diet to a high forage diet caused decreased E. coli fecal and rumen populations. Dried Distiller's Grains (DDG) are a byproduct feed linked to increased fecal STEC shedding. Influence of dietary composition and abrupt dietary shift to forages was examined in two live cattle experiments. Experiment 1 used n=32 Angus cattle (BW = 359 ± 62.2 kg) fed 0, 20, or 40% DDG for 35d before switching to 100% hay diet. Fecal E. coli counts were reduced (1.14 log10 CFU/g; $P < 0.001$) following the abrupt shift. However, cattle growth and feed efficiency were reduced ($P < 0.05$) and methane production was increased ($P< 0.05$). In Experiment 2, n=27 cattle were fed a starch-rich finishing ration for 21 d, followed by an abrupt shift for 22 d to: 1) high grain diet (control), 2) Low Quality Forage (LQF), and 3) High Quality Forage (HQF). By d29, fecal E. coli were decreased by 1.15 log10 CFU/g feces. By day 43, E. coli populations in LQF were lower than HQF group ($P < 0.05$), but not Controls. In both studies, fecal shedding of E. coli was negatively correlated with forage degrading bacteria populations and with Neutral Detergent Fiber (NDF) content of rations. Feeding finishing beef cattle on forage remains impractical logistically and economically, but that other NDF rich feedstuffs may be a tool to reduce fecal E. coli shedding.

Pop Up Sessions

Title: Post Infection Shedding

Date & Time: Monday 11/05, 1315-1415

Attendees: open to all VTEC-2026 conference attendees.

General description:
Current policies of post-symptomatic infection control for STEC usually exclude attendance at day care for children recovering from an acute symptomatic infection. This is based on early literature, in which such exclusion policies also included prohibition of attendance by children with acute diarrhea. The policy of excluding symptomatic children at the height of infectivity (high pathogen presence in stool early in illness, and copious production of stool as part of the illness) is sound. The exclusion of children in the post-symptomatic phase until they have been tested negative twice, causes much family hardship, and it is time to examine the value of this component of approach.

Topics to be considered:

1. Risk of acquiring a clinically actionable secondary infection from an asymptomatic/post symptomatic excreter of a high risk STEC
2. Enforceability of these policies
3. Value of detection methodologies

Panel members:
From public health agencies from different geographical regions.

Regulatory Round Table discussion: Pathogenic E. coli – evolving definitions and effect on policy

Facilitator: Dr. David Goldman
Panelists: Professor Delphine Sergentet, VetAgro Sup (France),
Dr. Matt Gilmour, Chief Science Officer, Quadram Institute (England),
Dr. Glenn Tillman, Biological Information Specialist, US Department of Agriculture, Food Safety and Inspection Service,
Dr. Alex Gill, Research Scientist, Health Canada, Bureau of Microbial Hazards,
Dr. Kate Thomas, Senior Adviser (Microbiology), New Zealand Food Safety at the Ministry for Primary Industries .

Abstract:
Food safety regs are informed by the latest settled science in order to both maximize public health protection and to minimize cost to industry and reduce unnecessary food waste (due to false positives or overly broad definitions of the pathogen). The quest to increase knowledge of pathogens is continuous--science is not static, and new scientific discovery challenges policymakers to know when to update regulation.

STECs are responsible for more than an estimated 1M illnesses and 128 deaths each year worldwide. With the growing food insecurity, it is imperative to maximize our food production and distribution systems. Like most commodities, economics define the international trade in food commodities like beef, beef products, and produce. Public health agencies across various countries are continuing to monitor STECs in food products as well as illnesses attributed to STECs. All these data are continually shaping the knowledge spectrum for STECs and providing new insights. Furthermore, evolving science of pathogenicity and virulence are challenging regulatory agencies globally to meet their public health mandates without significantly adversely affecting global food supply and food security. This roundtable will bring together recognized experts from various public health and regulatory agencies to discuss and answer questions on 1) how current regulations are contributing toward STEC control; and 2) how evolving scientific data is shaping the ongoing and future of these regulations.

Microbiological criteria of foods usually consider target microorganisms defined by taxonomical characteristics (e.g. genus, species, serotypes). These definitions are impacting regulations, control plans, HACCP and analytical methods. However, in the case of STEC, national and international organisations (e.g. FAO/OMS, EFSA, CODEX, ANSES...) are proposing new risk profiles moving from the classical serogroup definition towards virulence gene profile. These new definitions may

have an impact on future regulations, food industry risk management and analytical approaches.

The roundtable aims to discuss around how scientific, risk-based approaches may be applied to define microbial criteria for STEC risk management considering the broad range of food products potentially impacted and public health protection. Speakers will present practical examples from different regulatory approaches and discuss how food industry could establish microbiological specifications for STEC based on risk profile assessment (e.g., type of food, raw materials, ready to eat, consumers' consumption habits, population risk, etc.).

Some questions to be addressed in this roundtable:

Understanding pathogenicity and virulence allows regulators to increase precision in regulation development. Can precision diagnostics lead to precision regulation?

How have the speakers' regulatory authorities incorporated the use of genotypes into their STEC regulations?

What other considerations, aside from pathogenicity and virulence, are there in refining STEC regulations?

Workshop

Towards an Achievable Toolkit

As part of its cross-sector initiative Advancing STEC Diagnostics, the Food Safety Research Network (FSRN) is co-organising a focused workshop at the 11th International Symposium on Verocytotoxin producing Escherichia coli (VTEC2026) in Aberdeen. This session will convene international experts, public health agencies, diagnostic developers, UK regulators, and food businesses to explore practical, risk-informed approaches to STEC testing across food, clinical, and environmental settings.

The FSRN's ongoing STEC workstreams have been gathering real-world insight into current testing availability, terminology and interpretation challenges, and unmet needs for UK businesses and laboratories. This workshop presents an opportunity to reflect on those learnings to date, benchmark them internationally, and discuss a shared direction of travel. A central goal will be to explore the feasibility of transitioning from generic E. coli indicators, still common in food testing, toward more specific STEC diagnostics that are scientifically robust and operationally feasible.

Flash Talks and Posters

Time-Course of Platelets Count in Children with Hemolytic Uremic Syndrome. Data from the ItalKid-HUS Network.

MD Daniele Rossetti, MD Letizia Dato, MD Giacomo Tamburini, MD Laura Daprai, MD Dario Consonni, MD Maria Cristina Mancuso, MD Gianluigi Ardissino

[1] Pediatric Nephrology, Dialysis And Transplantation Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico,Milan

Background:
Hemolytic uremic syndrome (HUS) related to Shiga toxin-producing Escherichia Coli (STEC) infection is a severe, life-threatening thrombotic microangiopathy (TMA). Endothelial damage causes platelet consumption and consequently platelet count is a key biomarker for monitoring disease activity. The present paper describes the time course of platelet count in a cohort of patients with STEC-HUS in order to provide references helpful in identifying subjects who divert from the expected course because complications.

Methods:
All children treated at our Center with a well-established diagnosis of STEC-HUS during the period 2010-2025 were retrospectively enrolled and platelets count was recorded until discharge. The nadir of platelets count for each patient was used to align the results and investigate the time course towards platelets normalization. The cumulative percentage of platelets normalization was calculated on daily basis, together with the 10th centile of platelets count.

Result:
During the 16 years under investigation, a total of 148 children were enrolled. The nadir of platelets count was 23,000/mm3 (IQR 14,000-39,000) on day 8 since the beginning of symptoms. By day 14 from the nadir, 100% of patients had reached a platelets count > 150.000/mm3.

Conclusions:
Given that STEC-HUS is a self-limiting disease, the related TMA is expected to spontaneously cease within a defined time. If platelets count has a different course from the expected, the patient is likely to have complications that need to be actively searched for and treated accordingly (e.g. atypical HUS, disseminated intravascular coagulation or heparin induced thrombocytopenia).

Serological patterns of IgM and IgG responses against prevalent STEC serogroups in an endemic setting: A retrospective study in Argentina

Biochemist Cynthia Maiztegui[1], Biochemist Carla Schesi[1], Technician Ariela Baschkier[1], Biochemist Elizabeth Miliwebsky[1]

[1] National Institute of Infectious Diseases - ANLIS “Dr. Carlos G. Malbrán”

In Argentina, STEC infections are endemic and represent a public health concern. Although laboratory diagnosis is based on the isolation and characterization of STEC strains, indirect methods of diagnosis such as the detection of anti-E. coli lipopolysaccharide (LPS) antibodies by glicoIELISA are used to complement conventional diagnostic techniques and enhance diagnosis sensitivity. Despite its diagnostic relevance, the temporal persistence and levels behaviour of IgM and IgG anti-LPS antibodies remains incompletely characterized. Therefore, a retrospective study was conducted between 2018 and 2025, including patients (n=78) with at least two available serum samples, to evaluate the persistence of IgM and IgG antibody levels against E. coli O157, O145, O121 and O103 LPS, the most prevalent infecting serogroups in Argentina. The Percentage of reactivity was analyzed in relation to the difference in days between symptom onset and sample collection, revealing four distinct patterns. To explore possible associations between reactivity patterns and clinical and epidemiological variables, descriptive statistics were combined with exploratory machine learning techniques, including Random Forest analysis to estimate the relative importance of age, time between samples, sex, location, and serogroup. The results were inconclusive but suggests that specific variables such as age and serogroup may be linked to development of certain serological profiles. This study represents an initial step toward a better understanding of the serological behavior of STEC infections. The identification of distinct patterns highlights the complexity of the humoral response to STEC and underscores the importance of serological studies to improve results interpretation, diagnostic support and epidemiological surveillance.

Enterococcus faecalis activates enterohemorrhagic E. coli virulence by increasing extracellular adenine concentration

PhD Thibaut Rosay[1]

[1] University Of Wisconsin- Madison

Enteric microbiota confers numerous advantages to the host, its diverse membership confers protection against colonization by pathogens and opportunistic bacteria. However, pathogens regulate their virulence repertoire to adjust to their niche within the host. Here we show that the pathobiont Enterococcus faecalis promotes enterohemorrhagic E. coli (EHEC)'s virulence. We report that metabolically active E. faecalis secretes a metabolite that enhances expression of the EHEC's type three secretion system (T3SS) and attaching and effacing lesion formation on cultured epithelial cells and human colonoids. Targeted and untargeted metabolomics both in vitro and in the presence of colonoids showed an increase in products of the xanthine-hypoxanthine pathway that results in adenine biosynthesis, and in vitro extracellular adenine concentration is increased in EHEC and E. faecalis cocultures. We observed that adenine promotes expression of the T3SS. Moreover, comparison of the transcriptomes of EHEC cultivated in presence of E. faecalis also depict an increase in genes involved in organic transport, more specifically, adeP that imports adenine. An EHEC adeP knock-out is not responsive to E. faecalis, confirming the role of adenine on EHEC virulence expression. By screening a transcriptional regulator knock out library, we identify that adenine acts through Hha. Adenine relieves Hha repression on the transcription of the genes encoding the T3SS. We show here that microbiota produced purines (adenine from E. faecalis in this study) are perceived by EHEC as signals helping them to successfully colonize their host. This study highlights the complexity of pathogen-microbiota-host interactions in the gut.

An emerging LEE-negative Shiga toxin–producing Escherichia coli strain caused severe Hemolytic Uremic Syndrome outbreak exclusively in adults in France

Dr Justine de Larminat, Mr Kevin La, Dr Philippe Bidet, Pr André Birgy, Pr Patricia Mariani, Mme Sandrine Liguori, Mr Pierre Phlipaux, Mme Celine Courroux, Dr Florence Crombe, Dr Jorge Blanco, Dr François Xavier Weill, Dr Carolina Silva Nodari, Dr Gabrielle Jones, Pr stephane Bonacorsi, Dr Aurelie Cointe

[1] Hopital Robert-Debre, Service De Microbiologie, National associated Reference Center for STEC

Background:
Stx-producing Escherichia coli (STEC) infection is the leading cause of hemolytic uremic syndrome (HUS) and kidney failure in children under 3 years old and more rarely in adults. Surveillance of STEC in France is based on voluntary notification of pediatric HUS cases to Public Health France , combined with microbiological surveillance by the French associated National Reference Centre for E. coli.

Outbreaks documented to date have been largely limited to children and the major serogroups O26, O157, O80, O145, and O103. Between December 2024 and February 2025, we observed in France an unexpected rate of STEC HUS in adults caused by a singular STEC (LEE negative, major serogroups negative).

Main findings:
Whole-genome sequencing identified an outbreak strain as O77g:K92:H18, belonging to phylogroup D, harbouring the Shiga toxin 2 gene variant stx2d-073-C165-02 and carrying a 134kb plasmid with enterotoxin genes. The outbreak included 18 confirmed cases, and five probable/possible cases detected by in-house specific PCR, with two additional cases from Scotland and Belgium. Exclusively affecting adults (median age 72.1 years), the outbreak was unusually severe, with a HUS rate of 91%. Epidemiological investigations implicated a raw milk cheese as the contamination source.

Conclusion:
The strain represents a singular STEC-ETEC hybrid pathotype, harboring genes encoding a K92 capsule with a known cross-immunogenicity to Neisseria meningitidis group C, possibly explaining the absence of pediatric cases due to mandatory meningococcal C vaccination of infants since 2018 in France. Related strains have been identified in international databases since 2005, suggesting recent global emergence.

Sequence analysis of Stx2i-producing Escherichia coli strains isolated from raw lamb

Ms Noor Shubair[1,2], Dr Mathu Malar[1], Mr Austin Markell[4], Dr Alex Gill[3], Dr. Catherine Carrillo[1,2]

[1] Canadian Food Inspection Agency, Ottawa Laboratory (Carling), [2] Carleton University, Department of Biology, [3] Health Canada, Bureau of Microbial Hazards, [4] Canadian Food Inspection Agency, Food Safety Science Directorate

The definitive virulence factor of Shiga-toxin producing E. coli (STEC) is Shiga toxin. These toxins are classified into types (Stx1, Stx2) and further divided into subtypes (e.g., Stx2a through Stx2o). Specific subtypes are strongly associated with disease severity and host specificity. The Stx2i subtype is relatively rare and, in Canadian food testing programs, we have recovered it only from raw lamb products.

Given the potential association between Stx2i and ovine sources, we aimed to characterize the genomic diversity and virulence profiles of Stx2i-positive strains recovered from raw lamb and other sources to better understand their public health significance.

We analyzed seven STEC genomes from strains recovered from six raw lamb product samples along with 60 additional published genomes. The dataset comprised strains from 10 different countries recovered from sources including sheep/lamb, oysters, dairy, periphyton and human. Genomic analysis of the seven Stx2i-encoding STEC isolates recovered from raw lamb revealed significant serotype diversity: OUNT:H21, O8:H19, O8:H25, and O9:H30. Notably, two unique strains encoding Stx2i were recovered from a single sample, demonstrating diversity even within one source. Examination of the published genomes identified an additional five serotypes. Importantly, human clinical isolates of serotypes O100:H30, O8:H19, O8:H25, O8:H9, O9:H19, and O9:H30 were identified.

This study confirms that diverse E. coli strains encoding Stx2i are circulating globally in food, particularly in ovine sources. The overlap between serotypes found in these reservoirs and those recovered from human patients highlights the potential clinical relevance of stx2i-producing strains isolated from food.

PulseNet Latin America and the Caribbean: Strengthening the genomic surveillance for STEC non-O157 and emerging patotypes.

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Regional Network PulseNet Latin America and the Caribbean (PNLAC) conduct several initiatives as a regional roadmap to reinforce foodborne pathogen surveillance, in alignment with the PAHO genomic surveillance strategy. Surveillance of Escherichia coli in the region needs to be strengthened, as only few countries conduct routine diagnosis/surveillance. As part of regional improvement efforts, PNALC has implemented genomic analysis for Shiga toxin–producing Escherichia coli (STEC) O157, and STEC-regional database (hosted on ANLIS-Malbrán server and curated by Servicio Fisiopatogenia) containing 70 STECO157-sequences has been established. Currently, the analysis has been expanded to non-O157 STEC. The aim of this work was to determine the diversity of non-O157 STEC isolated in the region (2015-2024). A total of 130 sequences from Argentina (n=63), Paraguay (n=42), Perú (n=4) and Costa Rica (n=21), were analyzed with a command-line flowchart (FastQC, Kraken, MLST, Virulencefinder/Resfinder/AMRfinder_plus, Serotypefinder, Unicycler, Prokka, Roary). 39 serotypes/130 sequences were identified. STECeae(+) O145:H28, O26:H11, O103:H2, O111:H8, and STECeae(-) O113:H21, O8:H16, O71:H, were prevalent being the frequency variable depending on the country. STEC O48:H7 and O112:H2, not frequent serotypes, were detected in diarrhea in Argentina (sporadic cases and outbreak-associated) and Paraguay (sporadic cases). EAEC-Stx O59:H19 and O104:H4 were identified in Argentina. Pangenome analysis yielded a phylogenetic tree showing a great diversity and variability (stx, AMR and virulence genes); some strains clustered by serotype/virulence profile. In conclusion, we highlight the circulation of main serotypes and new patotypes in the region, and serotypes in common between countries. Continued strengthening of genomic surveillance is essential to improve response strategies.

Genomic Epidemiology of Shiga Toxin-Producing Escherichia coli in Argentina

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Nowadays whole genome sequencing (WGS) is a methodology more accessible for Public Health laboratories. Since 2015 the National Reference Laboratory (NRL) has started WGS implementation for surveillance of STEC infections and for foodborne outbreak investigation and in 2023 real-time diagnosis was successfully achieved. The aim of this work was to show results obtained during 2024-2025. STEC strains isolated in Argentina via HUS and diarrheal surveillance during January 2024 and October 2025 were sequenced using Illumina technology and analyzed with a flowchart based on command line scheme (FastQC, Kraken, MLST, Virulencefinder, Resfinder/AMRfinder_plus, Serotypefinder, Unicycler, Prokka, Roary, Snippy). Out of the total STEC strains studied (n=280) 89.5%, 48.5% and 24.4% were isolated from haemolytic uremic syndrome, bloody diarrheal, and diarrheal cases, respectively. More than 14 serotypes were confirmed being O157:H7 (61%) and O145:NM[H28] (22%) the most frequently isolated. Others, such as O48:H7, O91:H21, O111:H8, O151/O118:H2, O85:H4 and OgN31:H49 remerged at low frequencies as well as EAEC-stx O59:H19 and O104:H4 hybrid strains. Pangenome analysis yielded a phylogenetic tree revealing high genetic diversity, enabling strain clustering and demonstrating variability in stx, AMR, and virulence genes, some clustered according to serotype. It is observed that stx2a, stx2c, eae, ehxA (85%) is the most frequent among STEC O157:H7 strains. Several outbreaks were studied by SNP analysis, mainly associated with STEC O157 and O145. WGS improved time response and fully characterization, and therefore, provided a better understanding of the epidemiological situation to respond to Public Health in a shorter time, at the national, regional and global levels.

The two-component system QseEF represses expression of the locus of enterocyte effacement (LEE) in EHEC under anerobic conditions

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Background:
Enterohemorrhagic Escherichia coli (EHEC) is a Gram-negative pathogen that colonizes human colon, which causes bloody diarrhea and life-threatening complications such as hemolytic uremic syndrome (HUS). The major virulence factors of EHEC include a pathogenicity island termed locus of enterocyte effacement (LEE) and Shiga toxins.

Rationale:
LEE gene expression is regulated by complex regulatory systems including two-component systems (TCS), which consist of a histidine sensor kinase and a response regulator. Understanding how TCSs regulate LEE expression will help to identify potential targets to treat EHEC. Previously, we reported a TCS, QseEF, which regulates LEE expression in aerobic conditions.

Outcomes:
The intestinal environment is largely anaerobic. Here, we confirmed under anaerobic conditions that QseEF repress LEE expression in vitro. Transcriptional profiling, immunoblotting and fluorescent actin staining assays showed more LEE gene transcription, protein production and bacterial attachment in qseEF deletion mutants, respectively, compared to wild type. QseG is an outer membrane lipoprotein co-transcribed with QseEF, and potentially activates QseEF. We found that QseG also represses LEE expression, suggesting that QseEFG may function as a three-component system to regulate virulence. RNA sequencing results showed that expression of genes related to bacterial pilus formation and acid resistance are also affected by the QseEFG system, indicating an extensive effect of this system on EHEC virulence regulation.

Managing outbreaks of Shiga toxin-producing Escherichia coli (STEC) in childcare settings: lessons learnt and implications for future practice

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Background
STEC infections pose a risk of severe complications in young children, particularly haemolytic-uraemic syndrome (HUS). Managing STEC outbreaks in childcare settings can present challenges for public health professionals, requiring risk assessment by a multi-agency incident management team (IMT).

Rationale for the work
To share lessons learned from large-scale nursery screening exercises following two separate outbreaks of STEC O26 in South West England.

Main findings or outcomes
189 individuals were screened across both outbreaks (161 children and 28 staff). In the first outbreak, one confirmed and one probable case of STEC O26 were identified in symptomatic children; these could not be typed.

The second outbreak identified six cases of genetically identical STEC O26, and one case of STEC O146. Five cases were symptomatic, with one infant developing HUS; two asymptomatic cases were identified through screening.

Incidental gastrointestinal pathogens were detected in 60 individuals.

Environmental swabs from the first nursery, taken before deep cleaning, were negative for STEC. However, environmental swabs from the second nursery following initial deep cleaning were STEC PCR-positive on two occasions. IPC support was provided, and further deep cleaning conducted.

Conclusions
Large-scale screening exercises can identify additional asymptomatic cases of STEC in outbreak situations but are a significant logistical undertaking. They should therefore be considered following an IMT risk assessment. Environmental swabbing can indicate the effectiveness of cleaning, but results should be interpreted with caution. Robust IPC support is essential for an effective response, but clear roles and responsibilities for IPC in childcare settings require further development.

STEC in dairy calves: occurrence, genomic characterization and public health concerns

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Cattle are recognized as the primary animal reservoir of Shiga toxin–producing Escherichia coli (STEC), a transient member of their normal gut microbiota. In cattle, the pathogenic role of STEC has been demonstrated only in young calves with diarrhea. This study aimed to investigate the presence and genetic diversity of STEC in calves.

Fecal samples were collected from 198 diarrheic and non-diarrheic calves up to seven days of age from 15 Slovenian dairy farms. All samples were plated on Drigalski agar, and E. coli isolates were confirmed by MALDI-TOF. Up to three colonies with different morphologies were selected from each sample, and a total of 574 E. coli isolates underwent whole-genome sequencing. Virulence genes were identified using VirulenceFinder v2.0.

Of all isolates examined, 25/574 (4.4 %) were identified as STEC, originating from 9/15 farms. Isolates from the same calf that belonged to the same cgMLST cluster were excluded from further analysis. Of the remaining 21 isolates, 18 were stx1a-positive, while one isolate each was positive for stx1a/stx2a, stx2a and stx2c. The eae gene was present in 14/21 isolates. Isolates belonged to seven serogroups, with O103, O55, O26 and O111 being the most prevalent. Diarrhea was observed in 3/19 STEC-positive calves, providing no evidence for the association of STEC and diarrhea.

These findings confirm calves as a reservoir of genetically diverse STEC strains. The detection of three serogroups belonging to the top five associated with human infections further emphasizes the need for effective on-farm interventions to reduce the risk of STEC transmission.

Poster Presentations

Mitigating Shiga Toxin–Producing Escherichia coli Risk in Raw-Milk Cheese: A One Health Approach to Public Health Protection in Italy

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Background:
Shiga toxin–producing Escherichia coli (STEC) are zoonotic pathogens able to cause severe illness, death and sequelae. After serious paediatric HUS cases linked to raw-milk cheese consumption, in 2024 the Italian Ministry of Health requested a scientific opinion to the national public health institute on STEC risk posed by these commodities. In 2025 the Ministry appointed a multidisciplinary working group to support the development of mitigation policies against the STEC risk associated with raw-milk cheese.

Rationale:
Based on the scientific opinion, which compiled available evidence from Italy, Europe and the international literature the working group including physicians, veterinarians, microbiologists, epidemiologists and food technologists, representing clinical practice, diagnostics, public health and food safety operated with an integrated approach.

Outcomes:
Guidelines on STEC control measures in unpasteurized milk products were developed to support food business operators (FBOs) in managing microbiological risks along the dairy supply chain and to assist competent authorities in official control activities. The guidelines were accompanied by recommendations to strengthen STEC surveillance in public health through improved early diagnosis and integration of clinical, epidemiological, and genomic networks.
Experts also highlighted the importance of intersectoral training and communication to improve FBOs and public engagement and recommended preventive labelling for at-risk cheese to promote informed consumer choices.
Guidelines and reccomendations underwent key stakeholders consultation including patients, producer, and consumer associations.

Conclusions:
Italy is among Europe's leading high-quality cheese producers and the initiative represents a practical example of applying an integrated One-Health approach and underscores the need for structural risk management strategies.

The Food Standards Agency STEC One Health Framework

Dr Anthony Wilson[1], Dr Amy Hale[1], Dr Katy Rosser[1]

[1] Food Standards Agency

Shiga-toxin E.coli (STEC) is a re-emerging public health issue, with the rise of new strains exacerbated by climate change. There is an urgent need for research to better understand and formulate effective interventions. In this poster we will summarise the FSA's current programme of work to understand exposure pathways through environmental surveillance and better understand and manage the risk to UK consumers from STEC.

Developing national public health guidance for STEC: Scotland's story

Mr Colin Sumpter[1], Mrs Lisa Gardiner[1]

[1] Public Health Scotland

The national public health guidance for Shiga toxin-producing Escherichia coli (STEC) in Scotland was updated in early 2025. Our presentation describes the process of guidance development, explains the evidence used to inform the recommendations and summarises facilitators and barriers to STEC guidance development. This will support other countries and regions undergoing guidance update.

This process was facilitated by the Public Health Scotland (PHS) Guidance Team. A multidisciplinary Guidance Development Group (GDG) was convened to develop the guidance. The GDG had expertise from national and local public health teams, microbiology, and environmental health. The GDG identified key questions to address in this update, and conducted an evidence review. Evidence included primary literature, international guidance, novel Scottish epidemiological analyses, and expert opinion. The evidence review particularly focused on infection virulence. A 'considered judgement' process was facilitated by PHS Guidance Team. This process moves the GDG from evidence to recommendation by assessing the strength of evidence, but also the contextual relevance to Scotland - including both harms and benefits of recommendations. An equality impact assessment was completed to assess and mitigate any direct or indirect effect on health inequalities. Draft guidance underwent stakeholder consultation, and materials were developed to support implementation of the guidance, including template letters and self-audit criteria. Publication followed accessibility guidelines.

An evaluation of the guidance development process was also undertaken with the GDG.

This rigorous, evidence-based, and collaborative process was followed to deliver guidance that is both accessible and able to be successfully implemented by users in Scotland.

The power of three: genomic, gene and phage variation of Shiga toxin subtype stx2a in STEC from England, 2016-2024

Miss Eleanor Hayles[1,2,3], Dr Ella Rodwell[4,5], Dr David Greig[5], Dr Claire Jenkins[5], Dr Gemma Langridge[1,3]

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Background:
Phage-encoded Shiga toxins (Stx), the hallmark feature of Shiga toxin-producing Escherichia coli (STEC), are linked to varying clinical severity. Stx subtype 2a (Stx2a) is associated with a higher risk of progression from foodborne gastroenteritis to severe haemolytic uraemic syndrome (HUS).

Methods:
19,429 E. coli genomes submitted to UKHSA between 2016-2024 were extracted from EnteroBase, with serotype and stx gene information per isolate obtained from UKHSA and additional stx subtyping performed using StxTyper. Serotype distributions were quantified in R and visualised using Grapetree. Gene-level variation was identified using snp-sites, quantified with VCFtools and phylogenetic relationships inferred using IQ-Tree. Prophage regions were extracted using PHASTER and annotated using Pharokka.

Results:
Overall, stx2a was present in 40.0% (n=4114) of STEC isolates across 76 O serogroups, most commonly in O157 (n = 2274, 17.7%), O26 (n = 824, 6.4%) and O145 (n= 562, 4.4%), with increasing prevalence over time in unique non-O157 serogroups. stx2a was most commonly observed alone, in 18.4% (n=2375) of isolates, and was also present in 16 additional stx subtype combinations. Gene level analysis identified 89 SNPs with multiple allelic variants clustering by serotype, with additional diversity in further stx presence alongside stx2a. Phage-level analyses integrated with gene level and genomic context data demonstrate connections between serotype and stx2a variants.

Conclusion:
stx2a is prevalent within UKHSA STEC isolates, with multiple allelic variants and increasing acquisition amongst O serogroups, highlighting stx2a as an emerging public health concern. The study also emphasises the value of genomic surveillance within public health.

STEC contamination in kosher beef: A traceability-based approach to food safety

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Cattle are the main reservoir of Shiga toxin–producing Escherichia coli (STEC), and beef contamination occurs mainly during slaughter. However, information on STEC in alternative slaughter systems, such as kosher production, remains limited. This system presents specific operational characteristics, including a mandatory salting process, which may influence contamination patterns along the production chain. Carcass sponge samples were collected from four slaughterhouses, before and after salting, ensuring hock-level traceability, together with beef samples obtained from five retail outlets. Of a total of 88 carcass sponge samples, 25 were potential STEC-positive, with an uneven distribution among slaughterhouses (12, 7, 5 and 1). From these samples, nine STEC isolates were recovered. Eight isolates originated from a single slaughterhouse, whereas the remaining isolate was obtained from a facility with one potential positive sample. Three isolates were detected in pre-salting samples and six in post-salting samples, with persistence of the same isolate observed across the process in one case. All isolates carried the stx2 gene. Of the five retail beef samples, only one was potential positive, with no confirmed isolate recovered.

The study of a poorly characterized matrix such as kosher beef provides relevant evidence on the presence and behavior of STEC in alternative slaughter systems. These findings contribute to the identification of critical processing stages and support traceability-based control strategies within a production and public health context where information remains limited.

A comparative analysis of phenotypic and genetic characteristics of biofilm formation in Escherichia coli O157:H7 harboring the stx1 gene

Vinicius Silva Castro[1], Aaron de Silva[1], Rees Wilson[1], Professor Kim Stanford[1]

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Biofilm production in E. coli O157:H7 is debated; some studies report minimal biofilm formation, possibly because the stx1 prophage occupies the mlrA region and impairs curli production. In this study, we analysed twenty-five O157:H7 strains carrying stx1 (with stx2 facultative) for their ability to form biofilms in 96-well polystyrene plates and compared these findings with whole-genome sequencing data. Analysis of stx subunits, the locus of stx insertion, and comparison with 201 genes previously associated with biofilm formation were performed. Of the 25 strains, only one was a biofilm-forming strain. Although all strains harbored the stx1a gene, only 24% carried stx1a alone, while 64% additionally carried stx2a, and 12% carried stx2c. Regarding stx insertion, 68% (17/25) mapped to yehV/mlrA site, 4% (1/25) to torS, and 28% (7/25), including the single biofilm-positive strain, to non-classical sites. Comparing biofilm-associated genes, we identified 19 genes related to biofilm formation in the biofilm-forming strain, but none of these genes were exclusive to it. However, an interesting finding was a single nucleotide polymorphism (SNP) in the zapE gene at position 307bp, was observed only in the biofilm-forming strain, which has been associated with biofilm induction in Pseudomonas. This finding is noteworthy given the close genetic relatedness between the biofilm-positive strain and a non-biofilm-producing strain, which differ by only 35 SNPs. Although the results reported here indicate a biofilm-forming strain with an intact mlrA site, other non-biofilm-forming strains exhibited the same profile. This finding highlights the high complexity of determining the biofilm phenotype using whole-genome sequencing alone.

Anthropogenic Water Contamination and Detection of DEC Strains in Southern Patagonia

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Diarrheagenic Escherichia coli (DEC) strains are transmitted via the fecal–oral route, typically through the consumption of contaminated water or food. Mortality from water-associated diseases exceeds 5 million people per year. In Tierra del Fuego (TDF), disease rates (hemolytic uremic syndrome and diarrhea) are high. We assessed the presence of water contamination in Ushuaia, where population has expanded into informal settlements located uphill from the city. We hypothesized that untreated drinking water represents a source of infection by DEC strains. Sampling campaigns were conducted in November 2021 and January 2024. A total of 145 water samples were collected from water sources located upstream and downstream of the settlements, and 27 Moore swabs were deployed. Samples were processed to detect genetic markers of DEC strains. Six potential positives for Shiga toxin–producing E. coli (STEC) were obtained, although none could be confirmed through isolation or qPCR. Additionally, 6 potential positives for enteropathogenic E. coli (EPEC), 13 for enteroaggregative E. coli (EAEC), and 2 for enterotoxigenic E. coli (ETEC) were detected. One EAEC strain was isolated from river water in a sampling point downstream of the settlements, and it was sequenced for characterization (serotype O99:H10, sequence type 34, phylogroup A, aggR+/ aaiC+).

The detection of DEC markers occurred at sampling points located downstream of the settlements and at city level, suggesting water as a source of anthropogenic contamination. Within the human–animal–environment interface, studying environmental strains carrying pathogenic genes may help elucidate their origin, transmission pathways, and implications for public health.

Serotype-Specific Genetic Diversity in STEC: Implications for Cluster Definition Thresholds in Outbreak Detection

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Background
Genomics surveillance of STEC is important for the detection and source-tracing of outbreaks. The setting of a single genetic distance threshold for cluster definition is challenging with pathogens characterized by large difference in genetic distances, like encountered between different serotypes of STEC.

Rationale for the work
In this study we investigated the genetic diversity within and between different STEC serotypes in the Netherlands, to enable establishment of evidence-based serotype-specific cluster cut-offs.

Main findings
Here we constructed allelic distance matrices from the cgMLST profiles of a large dataset of STEC isolates for the most prevalent serotypes (O157, O26, O146, O103, O91, O63, O145) of which more than 50 samples were present in the national surveillance database since 2017. Within serotype-diversity ranged from median 495 (IQ: 375-826) alleles difference for serotype O63 to median 1107 (IQ: 717-2971) alleles difference for serotype O145. For O157 the median was 791 (IQ: 566-1060), 1070 (IQ: 661-3012) for O146, 809 (IQ: 544-1140) for O103, 1062 (IQ 811-1362) for O91, and for O26 the median was 679 (IQ:525-972) In addition, we observed a bimodal diversity within most of the major serotype groups, with the exception of O63, with a smaller sub-group of isolates showing much higher (2500-3000) genetic distances. This is highlighting an extreme divergence even within serotypes.

Conclusions
The width of genetic diversity differs considerably between the most prevalent STEC serotypes. Consequently, a common fixed cluster detection threshold may not be the most suitable approach for outbreak detection. We propose serotype specific thresholds for STEC.

Genomic characterization of stx2k-positive Escherichia coli isolates from food samples and a human case in Italy

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Background
The stx2k subtype has so far been identified in isolates from China and South Africa. This study provides the characterization of stx2k-positive E. coli strains isolated from food and human samples from Italy.

Rationale for the work
Two strains were isolated from raw milk and curd sampled in 2015 and one from a diarrheal patient from 2022. The genomes were sequenced with short and long reads technologies and analysed with PHANtAsTiC pipeline for serotyping, virulotyping and cgMLST, and with MAUVE and Easyfig for characterizing stx2k-bacteriophages.

Main findings
The food isolates belonged to O100:H19, while the human isolate harboured fliC H21 allele and a wzx gene showing 89% identity with O155 allele. All the strains harbored the heat-labile enterotoxin–encoding gene.

The food isolates clustered with stx2k-producing strains isolated in China in the period 2013-2021 (≤51 AD), while the human isolate was closely related (6 AD) to a strain isolated in China in 2013 (STEC309).

The stx2k-bacteriophages were integrated at dusA in the food isolates and at yecE in the human isolate. The former matched the genetic structure previously described in stx2k-positive strains from China, whereas the latter showed a novel organization, sharing a 20 kb region (≥97% identity) with an stx-negative bacteriophage present in the genome of STEC309, harbouring structural genes.

Conclusions

This study reports the first isolation of stx2k-positive strains in Italy, highlighting the circulation of persistent clones across countries and suggesting that phage recombination may foster the emergence of novel mosaic phages capable of spreading stx2k genes.

A six-year genomic surveillance of STEC infections in Belgium, 2019-2024

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Genomic surveillance of Shiga toxin-producing Escherichia coli (STEC) through whole-genome sequencing (WGS) is the state-of-the-art tool to identify STEC clusters and to fully characterize circulating strains enabling risk assessment for haemolytic uraemic syndrome (HUS) development. On top of this, WGS facilitates national and international data exchange. From 2019 to 2024, the Belgian National Reference Centre (NRC) performed WGS on all STEC isolates including only one isolate per familial cluster.

Over this 6-year-period, the NRC identified 1235 isolates linked to 1221 individuals. A sustained increase in STEC was observed and particularly in non-O157 (2019: 57/123; 2020: 39/81; 2021: 69/118; 2022: 106/179; 2023: 257/330; 2024: 321/404). In total, 849 non-O157 isolates were detected, encompassing 84 different serotypes. The most common non-O157 serogroups were O26 (16.6%), O103 (8.1%), O63 (9.0%), O145 (8.4%) and O146 (7.5%). Most isolates were associated with a high (34.4%) or medium HUS risk (34.4%), which was even more pronounced among HUS-associated isolates, for which the majority exhibited a high HUS risk (82.3%). Overall, 23.9% of the isolates were classified into 98 molecular clusters.

In conclusion, the current surveillance system provides an enhanced understanding of circulating STEC isolates in Belgium. Prioritizing high-risk isolates will help public health authorities focus efforts on patients infected with the most pathogenic STEC.

Epidemiological trends of Shiga-toxin producing Escherichia coli (STEC) O157 in England 2016-2024

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[1] UK Health Security Agency

Shiga-toxin producing Escherichia coli (STEC) serogroup O157 is a well-known cause of infectious gastroenteritis in England. Cases can develop complications such as haemorrhagic colitis and haemolytic uraemic syndrome (HUS). Despite widespread adoption of polymerase chain reaction (PCR) testing in local laboratories for detection of STEC non-O157, STEC O157 remains the predominant single reported STEC serogroup.

We describe the epidemiology of STEC O157 infection in England between 2016 and 2024. Demographic, clinical and microbiological data were extracted from the National Enhanced Surveillance System for STEC (NESSS).

The number of STEC O157 cases reported decreased from 2016 (n=663) to 2021 (n=364), before peaking in 2022 (n=761) and subsequently decreasing below this peak (2023, n=532; 2024, n=563). Incidence of infection remains highest in children under 5 years. There continues to be a distinct seasonality with cases peaking in July to September. Heterogeneity among stx subtypes has increased, with a higher proportion having a combination of stx1 and stx2 (2016, 26.4%; 2024, 45.7%), and a lower proportion with stx2 only (2016, 73.2%; 2024, 53.9%). Consistent with this is a marginal decrease in the rate of hospitalisation, down 1.9% across the same period; the number of cases developing HUS have remained broadly stable. Across all years, the number of genomically-linked, common source clusters have remained similar. Finalised data from 2025 is expected to continue these trends.

Integration of epidemiological, genomic and microbiological surveillance is essential to continue understanding the changing burden of STEC O157 infection, inform ongoing public health risk assessments and mitigating foodborne outbreaks.

Detection of Shigatoxin-producing E.coli (STEC) in flour – recap of a proficiency test

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In the last years, triggered by several STEC-outbreaks in northern America and France flour came into spotlight of research and diagnostics. Since efficient microbiological diagnostic procedures are a prerequisite to generate trustful and comparable data on prevalence, survival and contamination level of STEC in flour. As a reference laboratory, we conducted a proficiency test focussing on detection and isolation of STEC in flour to compare laboratory performance, generate knowledge and determine the efficiency of the used methods for this rather dry food matrix. We used a set of nine samples consisting of two times four samples inoculated approx. 59 CFU and 9 CFU per sample, respectively and one plain flour sample as negative control. Twenty-eight Laboratories analysed these samples by either a protocol according to national standard (64 LFGB, L 25.00-6) originally validated for fresh produce and/or other methods (like ISO/TS13136:2012). Overall detection of STEC leads to successful isolation in over 90% of samples. However, the limit of detection (LOD_{50}) was calculated to be approx. 26 CFU per sample, whereas the method L 25.00-6 performed slightly better. Noteworthy, the usage of antibiotics and bile acids in the enrichment broth hindered STEC detection in the proficiency test. This implies that laboratories should consider method adaptation when investigating flour for STEC. Here methodological improvements like increased sample volume could lower the LOD, especially to detect the low contamination levels of STEC in flour. The further improvement of methods as well as method
standardization are the ongoing next steps.

Role of Urine dipstick and Azithromycin for the Management of an Outbreak of Shiga toxin-producing E.coli Infection, Italy, July 2025

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Background:
Shiga toxin-producing Escherichia coli (STEC) is a foodborne pathogen accounting for approximately 5-6% of cases of acute bloody diarrhea (ABD) in Europe. Herein, we report an outbreak of STEC infection involving a group of scouts, with 31 subjects exposed.

Rationale for the work:
The pivotal role of urine dipstick testing for hemoglobinuria (uHb) to identify patients with ongoing hemolytic uremic syndrome (HUS), as well as the potential benefit of azithromycin treatment to prevent the development of the renal complication, are described and discussed.

Methods:
All participants were invited to provide a stool sample for Stx-encoding genes testing. Symptomatic patients underwent urine dipstick for uHb and immediately started azithromycin (10 mg/kg/day). Patients who tested positive at urine dipstick (uHb≥2+) were further investigated with blood tests to rule in or out the diagnosis of HUS.

Outcomes:
Out of 31 exposed subjects, 26 were symptomatic and 13 tested positive for genes encoding Stx1, Stx2, and O157. None progressed to HUS.

Conclusions:
Based on this experience and recent scientific advancements, the key procedures for an effective management of STEC infection outbreaks are summarized in ten "hints and tips".

Should animals be sampled?

Ms Susan Neale[1], Dr Adrienne Mackintosh[2]

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When a human outbreak of Shiga toxin-producing Escherichia coli (STEC) occurs, a common question is ‘should animals be sampled?

For farm premises epidemiologically linked to the outbreak, the Animal and Plant Health Agency (APHA) specialist veterinarians provide advice to assist decision making within the Outbreak Control Team (OCT). For farm visitor attractions (involving direct/indirect contact with animals) an APHA advisory visit is recommended. If the implicated animal species are present, the decision for collecting and testing freshly voided faeces samples is made. Recommendations within the veterinary remit to reduce the risk of zoonotic transmission are provided to the keeper and communicated in summary to partner agencies within the OCT.

For food associated outbreaks other organisations provide specialist advice and perform food and environmental sampling, since failure of hygiene measures in the food chain has led to this route of infection. Animal sampling is often discussed, but may not be recommended, as detection of the outbreak STEC strain in the suspect food item is preferable. Irrespective of sampling results, the cause of the food contamination must be identified and advised on to mitigate future risks.

In appropriate circumstances animal sampling may assist outbreak investigations; however it is important that the limitations of sampling and meaning of test results are correctly understood. When animal faeces sampling is performed, detection of the outbreak STEC strain indicates presence on the farm; non-detection of the outbreak strain does not confirm the farm is not the source of infection.

Shiga toxin producing Escherichia coli O26: 48 years later...

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Background
In 1978, examination of Escherichia coli in the UKHSA archive found 25 isolates that produced Shiga toxin (Stx), of which 23/25 belonged to serotype O26:H11. Despite the early work on Shiga toxin-producing Escherichia coli (STEC) O26, STEC O157 subsequently emerged as the most important threat to public health in the UK, and surveillance algorithms focused on this type.

Rationale for the work
Recently, STEC O26 has re-emerged in England as a major public health concern. We aimed to review the laboratory surveillance data to explore the drivers of the increase in notifications and assess the on-going risk to public health.

Main findings or outcomes
Since systematic laboratory surveillance for non-O157 STEC was implemented in 2014, diagnoses of STEC O26:H11 has increased each year from 19 to 356 in 2024. Most isolates had the Stx subtype profiles stx1a (47%), stx1a/stx2a (24%) or stx2a (28%), with isolates harbouring stx2a exhibiting an increasing trend. Most cases were female (57%), and the highest proportion of cases belonged to the 0–5 age group (38%). The proportion of cases of haemolytic uraemic syndrome caused by STEC O26 has increased from 3.3% to 48.6%. Outbreaks have been linked to foodborne transmission, nursery school settings and petting farms.

Conclusions
STEC O26:H11 are a clinically significant, emerging threat to public health in England. Determining the true incidence and prevalence is challenging due to inconsistent national surveillance strategies. Improved diagnostics and surveillance algorithms are required to monitor the true burden, detect outbreaks and to implement effective interventions.

Fermented milk does not prevent the carriage of Escherichia coli O26:H11 in dairy calves

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The aim of the ConEHECtion project was to evaluate the use of milk fermented by Streptococcus thermophilus and Lactobacillus delbrueckii subsp. bulgaricus, as a supplement to a conventional colostrum/milk diet, in order to control gut microbiota and prevent STEC/AEEC colonisation in the digestive tract of young dairy calves in the raw milk sector.

A total of 56 calves born in four farms historically contaminated by STEC/AEEC O26:H11 were recruited. In each farm, 14 calves were divided into 2 groups, the control group and the fermented milk supplemented group that received a daily dose of fermented milk (containing more than 109 CFU of lactic acid bacteria). STEC/AEEC faecal carriage was assessed by taking faecal samples and swabs from the recto-anal junction four times between birth and weaning.

A logistical model studied the impact of fermented milk distribution and calf management on STEC/AEEC O26:H11 carriage.

62.5% of 56 calves were STEC/AEEC O26:H11 carriers at least at one sampling point. Calves aged between 1 and 2 months excreted the bacterium (32% and 35%, respectively) more often than calves immediately after birth (4%) or after weaning (68 to 148 d) (19%).

No difference was shown on STEC/AEEC O26:H11 excretion between calves that did or did not receive fermented milk (26% versus 19%). Fermented milk therefore did not help to control STEC/AEEC O26:H11 excretion in our experiment.

Further characterisation of the faecal microbiota of calves is ongoing to better understand the interactions between the intestinal microbiota and STEC/AEEC O26:H11.

To pasteurise or not to pasteurise: the diversity of Shiga-toxin producing E. coli (STEC) in Scottish dairy cattle herds

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In Scotland, mandatory pasteurisation is required of raw milk, or cream, that is intended for direct human consumption and placed on the market by any distribution route. However, cheese production from raw milk is legal in the United Kingdom (UK). These products can become contaminated with STEC.

In 2023/24 we collected faecal samples with the aim to investigate the diversity of STEC in Scottish dairy cattle.

In four commercial dairy herds, we obtained faecal samples from the milking herd (N = 164 samples). We also obtained samples from 288 healthy dairy cull cows, at slaughter. Using culture techniques with PCR confirmation of the presence of O157 and stx genes, O157 STEC were obtained in one of the milking herds. Diversity of non-O157 STEC, defined as the presence of different combinations of stx1 &/or stx2, varied between the herds both in frequency and types. The results from the cull dairy cows indicate that: while STEC are isolated at moderate frequencies, with just under half of positive for the eae gene; the most common stx type was stx1, while O157 STEC occur at a low frequency.

All isolates have been sequenced and analysis of the genome sequence data, which includes placing them into the context of Scottish human clinical cases, is ongoing. Our findings highlight the importance of ensuring adequate milk pasteurisation and exercising caution with other dairy products, to prevent potential transmission to consumers via faecal contamination of milk

Phenotypic and genetic characteristics of STEC O157:H7 strains recovered from humans in Uruguay

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Background:
STEC causes several human diseases, including HUS. The most recognized STEC serotype associated with HUS is O157:H7.

Rationale:
The outcome of human–STEC interactions depends on multiple factors, including the characteristics of the strains involved. This work aims to describe phenotypic and genotypic features of local O157:H7 strains.

Methodology and main findings:
Five STEC O157:H7 strains were characterized using biochemical tests, classical serotyping, disk diffusion assays, and PCR for the detection of selected virulence genes. Whole-genome sequencing (WGS) was performed for two strains, including determination of sequence type and cgMLST profiles, virulence-associated factors, and phylogenetic relationships. Three strains were isolated from adults sick (1 D, 2 UTI) and two from children (one with BD and one with HUS). All were sorbitol-negative, motile, and none showed antibiotic resistance. All carried eae variant γ-1, iha, and tir.

The distribution of stx genotypes was as follows: stx1 only (one strain), stx2 only (two strains), and both stx1/stx2 (two strains). Within stx1, variant a was identified in three strains; within stx2, three were variant a and one variant c.

Both sequenced strains belonged to ST11, cgMLST profiles 142741 and 17460, were predicted as human pathogens, sharing more than 20 virulence-associated factors. One strain (cgMLST142741) was phylogenetically close to other local O157:H7 strain of animal origin (cgMLST143316).

Conclusions:
These data suggest that this serotype is relatively stable in terms of sequence type and virulence genes (eae, ehxA, and stx1/stx2 variants), supporting its relevance for future studies on STEC O157 pathogenesis and development of potential immunogens.

Detection and Characterisation of strains carrying stx subtypes, stx2i, stx2k, stxl among STEC causing clinical infections in Scotland

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Shiga toxins (Stx) are the principal virulence factors of STEC and are responsible for the severe systemic complications associated with the infection. Surveillance of Stx in STEC is essential to evaluate their associated risk and ensure accurate detection. A Shiga toxin gene (stx) subtyping taxonomy was established in 2012 and PCR primers developed to detect the main subtypes stx1a,c,d and stx2a-g. Since then additional Stx (Stx1e, Stx2h, Stx2i, Stx2j, Stx2k, Stx2l, Stx2m, and Stx2n, Stx2o) have been described.

The aim of this work was to determine if the more recently described stx subtypes are detected by our WGS workflow, and to characterise the strains associated with carriage. The fasta sequences of 1410 non-O157 STEC collected over >20 year period were reanalysed with the BioNumerics Ecoli genotyping plug-in (version 2023.11.22), which contains reference sequences for all the stx subtypes. We detected seven isolates with stx2i and these belonged to serotypes O30:H25 (ST8660, n=4) and O100:30 (ST993, n=3). One isolate carried stx2k (O75:H5/ST404), while nine isolates had stx2l (O8:H19/ST162, n=4; O8:H9/ST23, n=3; O138:H48/ST219, n=1; O38:H45/ST13653, n=1). All these strains were negative for eae. No isolates were found to carry stx1e, stx2h, stx2j, stx2m, stx2n or stx2o, however, these subtypes may not be detected by our faecal screening RT-PCR.

Further work is ongoing to look at the clinical significance of the strains carrying these less common subtypes, and to determine if modifications are required to our workflow to ensure we are reliably detecting these strains.

Type B Private Water Supplies: To test or not to test? That is the question!

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Background
We present an unusual co-infection of non-O157 Escherichia coli alongside Campylobacter jejuni, identified from a single stool sample. The case lives on a working farm and relies on a private water supply. Both exposures were considered possible sources of infection.

Case presentation
A male in his early 40s with bloody diarrhoea and crampy abdominal pain tested positive for stx1 and stx2 positive non-0157 E. coli and Campylobacter jejuni. The case's two household contacts were not affected, and neither case nor contacts were in a risk group.

Collaboration between the Health Protection Team (HPT) and Environmental Health Officers (EHOs) revealed that the private water supply had no previous testing records. Initial sampling detected E. coli, leading to a boil water notice. Resampling after activating the UV filter again showed E. coli contamination. However, the samples were not sent to the Scottish E. coli Reference Laboratory (SERL) for typing, which could have clarified whether the water supply was the definitive source of infection.

Discussion
Although a typing result for the water sample would not have altered clinical management, it could have provided valuable epidemiological insight. Discussions with SERL confirmed their interest in receiving water samples positive for E. coli for genotyping in this context. We discuss the potential impact of this, and detail the working arrangements and responsibilities of the Environmental Health Department in this scenario.

Conclusion
This case resulted in discussions around standardising a process for similar future cases. Strengthening joint working between HPT and EHOs improves response and learning.

Validation of Gene Up® Ehec Method for Stec Detection Using New Molecular Markers and Isolation on Idea™ Stec Agar

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Gene Up EHEC method is a molecular method based on PCR. In order to reduce the number of false positive PCR results requiring confirmation, the method incorporated a new kit using molecular markers (Gene Up PEC kit targeting espK and espV genes) associated to colocalization of stx and eae genes in the same bacteria (included in the method USDA/FSIS MLG 5C.04). In addition, a new isolation agar using nanoparticles recovered with specific Shiga Toxin antibodies (IDEA™ STEC agar) has been proposed for TOP 5 STEC isolation targeting specific Shiga toxin production.

The objective of the study is to demonstrate the equivalence of the new workflow in comparison with the reference method ISO/TS 13136:2012 by following the criteria established in ISO 16140-2 for alternative method validation.

Preliminary results showed good inclusivity and exclusivity results for the 50 positive and 30 negative strains tested. Relative Limit of Detection (RLOD) comparing both methods with samples of ground beef meat (15% fat) spiked with STEC O103 (stx1+, stx2-and eae +) was RLOD = 0,290, below the maximum value accepted (RLOD ≤ 2,5), with values of LOD50 = 1,75 cfu/25g for the reference ISO/TS 13136:2012 and LOD50 = 0,40 cfu/25g for the alternative Gene Up EHEC method with both pre-enrichment times at 8 h and 24 h.

Sensitivity study on going is showing equivalent results when comparing both methods, demonstrating the good performance of the new markers and isolation agar when testing different meat samples spiked with STEC TOP 5.

Stitching genomics data to protein structures: Virulence factors in non-O157 Shigatoxin-producing Escherichia coli

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Background and rationale
The genetic diversity of the Shiga toxin-producing Escherichia coli (STEC) sub-species group poses major challenges for assigning pathogenic potential, and for accurate diagnostics. Although existing risk-based frameworks work for the predominant serotypes e.g. STEC O157 or O26, they are less useful for more diverse genotypes, with variation in the genetic complement of virulence factors that is responsible for disease outcome. Moreover, genetic diversity occurs at the virulence factor allele level.

Main findings
We investigated the functional potential of genetic variation at a whole genome level and at an allele variant level, based on the premise that genomic variability underlies phenotypic traits including disease outcomes. We analysed 286 non-O157 STEC genomes for their virulence factor complement and determined co-occurrence patterns. Genetic variation in the well-characterized virulence factor intimin (eae) and its receptor (tir) was analyzed at a sequence level, and by modelling the three-dimensional protein–protein complex using AlphaFold3 and homology modelling. Different subtypes of Eae were shown to preserve their interaction with the receptor Tir by retaining the interactions at the protein-protein interface.

Conclusion
Through this work, we would like to emphasise the importance of integrating genomic analysis with protein structural analysis which provides insights into mechanism of function, in this case pathogenicity of eae+ STEC isolates. The examples of co-occurring variations at protein-protein interfaces underscore the importance of integrating genomic data with structural biology to deepen our understanding of protein function.

Annexin-Apyrase fusion protein protects mice from enterohemorrhagic Escherichia coli-induced disease

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Circulating extracellular vesicles (EVs) deliver Stx2 to the kidney. Using an established mouse model of intragastrically-administered enterohemorrhagic Escherichia coli our group has previously reported that annexin-V and apyrase had a protective effect. Annexin-V increased macrophage uptake of EVs and decreased circulating vesicles. Apyrase decreased fecal Stx2 as well as bacteriophage DNA and protein levels. This study investigated the protective effects of an annexin-apyrase fusion protein in the EHEC mouse model.

Balb/c mice were inoculated with E. coli O157:H7 (86-24) and treated i.p. with APT402 (annexin-V-apyrase fusion protein), APT501 (annexin-V), APT102 (apyrase), or vehicle, daily during the first week, starting at three timepoints: 1h before or 24h or 48h after inoculation and followed for 14 days.

Mortality in mice treated 1h before infection with the fusion protein APT402 was significantly lower (n= 2/7) compared to vehicle (n=5/6, $P<0.05$). Significantly lower mortality was also seen in mice treated 24h after inoculation (n=1/10) compared to vehicle (n=7/10, $P<0.01$). Mice treated with APT402 showed significantly improved kidney function, measured by urea levels, and a tendency to reduced bacterial colonization on day 8 compared to vehicle. A similar protective tendency regarding survival and decreased colonization was seen in the infected mice treated with APT501(n=2/5) 24h after inoculation. Treatment with APT102 at all time points, and treatment with APT402 and APT501 at 48h after inoculation showed no protective effect.

This study demonstrates that the annexin-apyrase fusion protein was protective when administered 1h before or 24h after EHEC inoculation and should be further explored as a treatment.

Structural and dynamic insights into ribosomal P-stalk recognition by Shiga toxin 2 and ricin and inhibition of P-stalk interactions

Distinguished Professor Nilgun Tumer[1], Dr. Shibani Bhattacharya[2], Dr. Michael Rudolph[2], Dr. Xiao-Ping Li[1], Dr. Lina El-Sharkawy[1], Dr. Eric Bryan[1], Ms Gwen Kester[1], Mr. Benjamin Algawa[1], Dr. Zoltan Szekely[1], Dr. Jacques Roberge[1]

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Background

Shiga toxin 2 (Stx2) and ricin depurinate the sarcin/ricin loop (SRL) of the 28S rRNA after binding the conserved C-termini of ribosomal P-stalk proteins. Binding sites of both toxins are located away from their active sites. Disrupting toxin–P-stalk interactions with small molecules has emerged as a promising strategy to block an early and conserved step of intoxication.

Rationale

Small molecules targeting the conserved P-stalk binding sites of Stx2A1 and RTA can reduce ribosome recruitment and cytotoxicity. Yet structural comparisons show differences between the two pockets. Although both toxins depurinate the same target, inhibitors optimized for RTA often show reduced activity against Stx2A1, highlighting the functional relevance of those distinctions. Understanding how the pockets diverge in structure and conformational behavior is essential for developing broadly effective inhibitors.

Main Findings

X-ray crystallography revealed that RTA has a compact topologically constrained binding site, whereas Stx2A1 has a broader, more solvent-exposed interface that supports wider range of interactions. NMR-based chemical shift perturbation mapping of P11 peptide binding showed interaction patterns unique to Stx2A1. NMR relaxation studies further demonstrated that RTA's C-terminal pocket becomes more rigid upon peptide binding, while Stx2A1's pocket becomes more disordered, reflecting a more adaptable surface.

Conclusions

These results show that toxins use different allosteric strategies. RTA achieves catalytic efficiency by pre-organizing the active site, while Stx2A1 relies on conformational diversity. These divergent structural and dynamic responses explain the limited cross-reactivity of RTA-optimized scaffolds and emphasize the importance of toxin-specific interactions when designing P-stalk-targeted inhibitors.

More than 15 years of the concept of complement inhibition in E. coli-associated haemolytic uraemic syndrome (eHUS)

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Following Otto Götze's idea to detect C5, C6 and C7 on the surface of macrophages for biological functions, such as the "spreading" of these cells, Würzner and colleagues developed monoclonal antibodies directed against these complement proteins. Two anti-C5 (N19-8 and N20-9) and one anti-C6 (WU 6-4) were able to inhibit the terminal complement pathway. These studies of the years 1987 and 1988 were first presented at two International conferences in summer 1989, the first descriptions of inhibition of human complement C5 and C6 by monoclonal antibodies.

Later, this anti-C5 was sold by Otto Götze, Dr. Würzner's supervisor at that time, to Alexion. In the following months and years this antibody was a main focus of Alexion's studies on the inhibition of complement lysis, until they presented a "superior" monoclonal antibody, 5G1.1 which then served as matrix for eculizumab, Alexion's Soliris.

In Oxford and Cambridge Würzner continued his studies of complement inhibition by monoclonal antibodies and together with Dorothea Orth-Höller he discovered in Innsbruck that Shiga toxin activates complement and binds complement factor H more than 15 years ago. From this evidence for an active role of complement in E.coli associated hemolytic uremic syndrome (eHUS) they proposed that Eculizumab may be useful in severe forms of eHUS. In fact, this drug was then extensively used in the German 2011 EHEC outbreak. Today, use of Eculizumab in eHUS is still controversial, but favourable outcomes, especially in severe neurological involvement were reported. Recent meta-analyses and broad trials will be reviewed at the conference.

Susceptibility to azithromycin in STEC strains isolated from cattle

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Background:
Cattle are the most important reservoir for STEC, that are shed with faeces and can contaminate hide of live animals and, consequently, carcasses at slaughter. Currently, bovine meat and products thereof are among the most common vehicles for STEC transmission to humans. Recently, treatment with azithromycin of bloody diarrhoea has been suggested to prevent progression to HUS and current evidence support this strategy.

Rationale for the work:
STEC carried by cattle belong to several serotypes, including the most commonly responsible for human infection. In Italy, one hundred and fifty slaughtered cattle were tested for STEC on hides and carcasses in order to characterize the isolates by serotype and the main virulence genes associated with severe human disease, as well as to assess their susceptibility to azithromycin.

Main findings:
Twenty STEC isolates were detected from 6.7% of hides and 4.0% of carcasses. These isolates belonged to 11 serotypes, including O157:H7 and O26:H11. Whole genome sequencing revealed different virulence gene combinations: stx2c+eae (35%), stx2a+eae (20%), stx1a (20%), stx1a+eae (10%), stx1a+stx2a+eae (5%), stx1a+stx2c+eae (5%), and stx2g (5%). Susceptibility to azithromycin was detected in 90% of the isolates, and in 100% of those carrying stx1/stx2+eae. The MIC values for resistant and intermediate-resistant isolates were 32 mg/L and 16 mg/L, respectively.

Conclusions:
Even though most STEC carried by cattle at slaughter and transferable to beef can harbour the genomic determinants observed in STEC responsible for HUS cases in humans, their susceptibility to azithromycin seems promising for successful medical treatments.

Validating Thermal Inactivation of Outbreak Strains Using Naturally Occurring Escherichia coli O157:H7 in Compost

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Background:
Dairy manure is a major reservoir for Shiga toxin-producing Escherichia coli (STEC). Composting reduces pathogens and recycles animal waste into valuable by-products. However, some STEC-related foodborne outbreaks have been linked to raw or inadequately composted manure.

Rationale for the work:
Composting reduces pathogens, but survival of E. coli O157:H7 in real-world conditions remains uncertain. Controlled studies show rapid thermal inactivation yet reports of prolonged survival in manure piles raise safety concerns. This study verified whether outbreak strain data align with naturally occurring strains under composting conditions.

Major findings:
We compared thermal inactivation of 3 outbreak strains (OS) of E. coli O157:H7 with 3 naturally occurring strains (NS) by simulating early phase of composting process in fresh dairy compost. After temperature reached the target composting temperatures, samples were taken out and analyzed for surviving population by direct plating or enrichment. At 50, 55, and 60°C, NS survived for 19, 11 and 6 days in dairy compost, compared with 17, 9, and 4 days for OS strains. Based on thermal inactivation curve, the pathogen inactivation rates for both types of strains were about the same, however a few resistant cells of NS survived longer than those OS cells. The most heat-resistant strains were identified by pulsed field gel electrophoresis.

Conclusion:
Although NS and OS strains of E. coli O157:H7 showed similar inactivation rates during active composting, some NS cells persisted longer. To ensure compost safety, the finished compost should be tested for complete pathogen elimination using sensitive detection methods.

Cleaved and uncleaved forms of Stx2 in the pathogenesis of HUS

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Background
Stx2 circulates in the bloodstream of STEC-infected patients in uncleaved (intact A chain) and cleaved (A subunit split into two disulfide-linked fragments) form. Although both forms are equally cytotoxic, they differ in binding to human circulating cells, a crucial step in HUS pathogenesis.

Rationale for the work
We investigated the effect of cleaved and uncleaved Stx2 on the formation of Stx2-containing pathogenic extracellular vesicles (EV) derived from human circulating cells.

Main findings or outcomes
Human blood exposed to 2 nM uncleaved Stx2 released more EV (2.02×10^{11}/ml) than cleaved Stx2 (1.59×10^{11}/ml) or controls (1.31×10^{11}/ml), especially larger EV (>300 nm) (nanoparticle tracking analysis). Capillary Western blotting revealed increased expression of vesicular, platelet and leukocyte markers in uncleaved Stx2-EV compared to controls: Alix (175.5% ± 41.1, $p<0.05$), CD42a (188.0% ± 16.9, $p<0.001$), CD45 (155.0% ± 44.5, $p=0.099$). Cleaved Stx2-EV showed significantly lower expression of the same markers (Alix 16.8% ± 27.8, $p<0.01$; CD42a 19.6% ± 34.0, $p<0.05$; CD45 4.2% ± 7.4, $p<0.0001$) compared to uncleaved Stx2-EV (100%). Uncleaved Stx2-EV (3 µl) were more toxic to Vero cells than cleaved Stx2-EV (%viable cells 53.5% ± 1.6 vs 73.8% ± 9.4; $p<0.05$), despite free uncleaved and cleaved Stx2 showed equal toxicity when added to the same cells (IC50=0.232 or 0.202 pM).

Conclusions
Cleaved Stx2 generates lower amounts of pathogenic EV than native Stx2. The effect of the two forms of Stx2 in mice and whether combinations of cleaved and uncleaved toxins have additive or synergistic effects is currently being studied.

Haemolytic uraemic syndrome-derived stx2f-positive Escherichia albertii strain render commensal Escherichia coli pathogenic by lysogenic conversion

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Background

Hemolytic uremic syndrome (HUS) is most often associated with Shiga toxin (Stx)–producing Escherichia coli. Although certain Escherichia albertii strains harbor stx2f, infections with this subtype are typically mild, and its potential to cause severe disease has not been clearly established.

Rationale for the Work

We identified the first documented HUS case associated with a stx2f-positive E. albertii strain, JNE161089, isolated from a one-year-old child. Despite its extremely low Stx2f production in vitro, serologic evidence supported its etiologic role. This apparent paradox raised questions about JNE161089's ability to cause severe disease.

Main Findings or Outcomes

Whole-genome sequencing revealed an intact Stx2f-encoding prophage in JNE161089. Stx2f production was extremely low in JNE161089 monoculture but

substantially increased during co-culture with non-pathogenic Escherichia coli (E. coli) K-12 strain or in lysogenized K-12 derivative strains (K-12ø2f), which also exhibited strong cytotoxicity. In germ-free mice, JNE161089 alone caused no lethality, whereas infection with K-12ø2f or sequential infection with K-12 followed by JNE161089 strain resulted in significant mortality. Identical Stx2f prophages genomes in JNE161089 and K-12ø2f isolates from sequentially infected mice confirmed in vivo horizontal phage transfer.

Conclusions
Our results indicate that stx2f-positive E. albertii can cause severe disease not through its intrinsic toxin-producing capacity but through ecological amplification via prophage transfer to commensal E. coli. This study highlights the importance of pathogenic enhancement driven by bacterial–phage–microbiota interactions.

Oligomeric coiled-coil adhesins that drive chain-like adhesion diversify surface colonization strategies in Shiga toxin-producing Escherichia coli

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Background
Chain-like adherence pattern (CLAP) is an EibG-drived phenotype of certain Shiga toxin-producing Escherichia coli (STEC) lacking the locus of enterocyte effacement (LEE), but its dynamics and molecular basis are not well defined.

Rationale
Non-LEE-encoded adhesins are increasingly recognized as key determinants of STEC colonization and virulence. We therefore sought to resolve how CLAP develops over time, how it behaves under shear, and which adhesins and structural domains mediate this phenotype.

Main findings
Live-cell time-lapse imaging showed that chains originate from a single founder cell that undergoes successive non-separating divisions, forming elongated multicellular filaments. Under flow, chains resist detachment yet fragment at cell-cell junctions, releasing viable clonal units that disperse downstream while remaining surface-associated. Comparative genomic analysis of STEC from Japanese clinical isolates revealed substantial diversity among EibG-related adhesins and identified distinct lineages, including a previously unrecognized group of chain-like adhesins (Cla) that mediate CLAP but lack immunoglobulin-binding activity. Genomic context and structural modeling support evolutionary separation of Cla from canonical Eib-family proteins. Targeted mutagenesis mapped chain formation and IgG binding in EibG to discrete structural domains of this oligomeric coiled-coil adhesin.

Conclusions
CLAP represents a dynamic, surface-associated strategy that promotes persistence and clonal dispersal of LEE-negative STEC under shear. Diversification of EibG-related adhesins, including Cla, expands the repertoire of non-LEE adhesins contributing to colonization and virulence, with implications for risk assessment of LEE-negative STEC in clinical and food-chain settings.

Clinical and Epidemiologic Description of a Shiga toxin-producing Escherichia coli Childcare Outbreak

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Importance:
Shiga toxin–producing Escherichia coli (STEC) infections have significant morbidity, with 15-20% of young children developing hemolytic uremic syndrome (HUS) over half of whom require kidney replacement therapy (KRT).

Objective:
To describe the healthcare resource impact, clinical outcomes, and impact of daily laboratory monitoring for thrombotic microangiopathy (TMA) in a large point-source STEC outbreak.

Design:
Retrospective cohort study following exposure to a contaminated food item served to 11 childcare centres in Calgary, Alberta, Canada on August 29, 2023.

Setting:
Population-based cohort of outbreak-linked cases.

Participants:
Primary (N=326) and secondary (N=33), children (N=288) and adults (N=71), diagnosed with confirmed (i.e., positive nucleic acid amplification test and/or culture) STEC infection linked to the outbreak.

Exposure:
E. coli O157:H7 possessing genes encoding Shiga toxins 1 and 2.

Main Outcomes and Measures:
Healthcare utilization, symptoms and signs, occurrence of HUS, need for KRT, and performance of laboratory-based TMA screening.

Results:
90.8% (326/359) of infections were primary and 9.2% (33/359) were secondary. Asymptomatic excretion carriage represented 18.4% (66/359) of the cohort. HUS occurred in 7.4% (21/285) of infected children, all of whom had diarrhea, including 10.2% (21/206) of children with diarrhea. No infected adult developed HUS. This outbreak obligated 508 and 395 visits to an ED and a dedicated STEC-clinic,

respectively; 591 visits occurred during a 7-day period, starting six days after exposure. Forty children were hospitalized, 9 required KRT and 3 required intensive care. The most common symptoms in children and adults were diarrhea (91.2% and 80.0%), abdominal pain (62.4% and 55.0%), and bloody diarrhea (56.2% and 35.0%), respectively. TMA testing had 100% (95%CI: 83.9, 100.0) sensitivity, 95.7% (95%CI: 92.0, 98.0) specificity and a diagnostic accuracy of 96.1% (95%CI: 92.7, 98.2) for development of HUS.

Conclusions and Relevance:
This large point-source exposure led to intensive utilization of outpatient, ED, and inpatient resources and 21 cases of HUS, but a lower complication rate than in prior outbreaks and sporadic series. This outbreak highlights the value of a coordinated public health response integrated with clinical care, and how early identification of TMA might reduce or mitigate adverse outcomes.

Optimising public health response to STEC cases in the era of PCR panel testing: A process audit and options appraisal

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Background:
The introduction of PCR panel testing for all gastrointestinal samples in the South West in 2023 resulted in increased notifications of STEC PCR-positive, culture-pending cases to the regional health protection team (HPT). The absence of a consistent approach to managing these cases highlighted the need to balance risk with operational efficiency when taking timely public health action.

Rationale:
To understand the impact of PCR testing on STEC notifications and inform an options appraisal.

Methods:
Retrospective review of all STEC notifications from residents in the South West region between 01/07/2022 and 01/10/2024. Data on notification trends, PCR/culture correlation, diagnostic timelines, and laboratory communication were analysed over time using descriptive statistics. Detailed record review was undertaken on a random sample of 30 STEC notifications.

Main findings:
There was no substantial difference in total notifications one year before PCR implementation versus afterwards. 37% of PCR-positive cases were culture-negative with 5.1 working days between PCR and culture results, and ~10 days before reference laboratory confirmation. Record review highlighted frequent absence of clinical information to evaluate case definition in 73% of notifications and 85% of cases required HPT follow up of results from local labs and diagnostic databases.

Conclusions:
Delays in culture reporting and limited clinical information complicated risk assessment of PCR-positive, culture-pending STEC cases. Findings informed changes in STEC response protocols with the introduction of SMS only follow-up for culture-pending cases over 5 years old with no high-risk features. Re-audit underway to assess public health impact with results due in January 2026.

Food borne-outbreak analysis – direct identification of cluster strains in complex food matrices

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Isolation of Shiga toxin-producing Escherichia coli (STEC) from contaminated food matrices require extensive and time-consuming lab methods; however, in case of outbreak situations, it is necessary to identify a contamination source as fast as possible to prevent a further spread. Therefore, we investigated the application of outbreak cluster-specific PCR methods for complex food matrices to shorten lab hands on time and to identify the respective contamination source as fast as possible.

In our study, we contaminated mozzarella, raw milk cheese, alfalfa sprouts and mixed minced meat with two E. coli or STEC strains of which one strain was assigned as a human outbreak strain and the other as close related non-outbreak strain using two different spiking concentrations (104 and 10^2 cfu per 25g sample). Outbreak-cluster specific primer and probes were designed based on whole genome sequences to detect single nucleotide polymorphisms or the identification of unique core sequences of the outbreak-cluster.

Results of the outbreak cluster-specific PCR methods varied according to the spiking concentration, the incubation time, the tested food matrix and the applied PCR method. Best results were achieved for mozzarella cheese samples whereas raw milk cheese, alfalfa sprouts and mixed minced samples showed some unspecific binding in some of the applied PCR methods. Nevertheless, we were able to successfully applied at least one outbreak cluster-specific PCR method for each of the tested matrix. In total, outbreak cluster-specific PCRs are promising tool which needs further evaluation as the results seems to be food matrix and PCR-method dependent.

Optimisation of Rapid Multiplex Real-time PCR assay for Detecting STEC in Cattle faeces

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Background:
Shiga toxin-producing Escherichia coli (STEC) is a significant public health risk, with cattle serving as the primary reservoir. Detecting STEC in faecal samples is critical for outbreak control, but complex matrices often contain PCR inhibitors that reduce assay performance. Rapid, robust detection methods are essential for effective surveillance.

Rationale:
Our previous STEC PCR assays was only validated on pure cultures and have not been adapted for complex matrices. This study builds on our earlier real-time PCR screening by optimising a rapid multiplex real-time PCR assay using the QIAGEN QuantiNova Pathogen + internal control IC Kit for detecting STEC virulence genes (stx1, stx2, eae) and key serogroups (O26, O157, O55, O103, O111, O145) in enriched cattle faecal broth. Three DNA template preparation methods were evaluated for compatibility and efficiency: (i) slow centrifuged enriched freshly culture broth boiled for 15 minutes, (ii) PrepMan Ultra reagent, and iii) InstaGene matrix . Preliminary validation included E. coli laboratory strain of the target serogroups, non-target strains, and faecal samples spiked with laboratory strain of the target serogroups. Performance metrics for PCR included target sensitivity, specificity, limit of detection (LOD), and Interna Control (IC) consistency.

Main finding:
Initial results from our studies demonstrate successful amplification of all targets under rapid cycling conditions, completing in 42 minutes, with comparative analysis of LOD and specificity in progress.

Conclusion:
The optimised multiplex PCR assay, combined with effective DNA preparation, shows promise as a robust method for STEC detection in complex faecal matrices.

EHEC surveillance on a federal level – One Health approach to monitor and detect EHEC outbreaks in Bavaria (Germany)

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Bavaria, the largest federal state in Germany, is divided into 71 counties and 25 independent cities, each having its own local public health department. The state consolidates its scientific authority for health, food safety and animal health in a single, superordinate institution: the Bavarian Health and Food Safety Authority (LGL). This focus ensures the coordinated surveillance of outbreak events.

To obtain an overview of the EHEC strains circulating in Bavaria and to be able to monitor, investigate, and contain possible outbreaks, all EHEC positive human isolates are subjected to whole genome sequencing (WGS). Since 2024, following the one health approach, additionally all food-associated, as well as other environmental EHEC isolates, are analyzed accordingly, to ideally identify the underlying source of detected outbreaks. Especially when a recognizable epidemiological link exists, WGS offers a powerful tool to scientifically support appropriate public health measures for outbreak containment. This is showcased via an EHEC outbreak that occurred in connection with various Bavarian daycare centers in the summer of 2023.

Besides the subtyping of virulence factors (e.g. Shiga-toxin genes), core genome multilocus sequence typing (cgMLST) is performed and cross-checked with an LGL-internal database. An additional cross-institutional and cross-state comparison is done via the Microbial Genomic Surveillance (miGenomeSurv) consortium. The initiative, comprising different public health stakeholders, aims to support Germany-wide, integrated genomic surveillance for various recognized high-priority pathogens, including EHEC. This aids the detection of larger multi-state outbreaks and provides a platform for direct communication with other consortium members.

The impact of PCR diagnostics on STEC detection, characterisation and severity assessment

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Background
The epidemiology of Shiga toxin-producing Escherichia coli (STEC) is increasingly shaped by advancing diagnostics. The introduction of PCR testing within one Scottish health board enhanced detection of strains previously missed by culture-based methods. This study examined the factors associated with more severe clinical STEC outcomes.

Methods
A retrospective analysis was conducted on STEC cases detected via routine PCR testing within NHS Tayside (April 2022-March 2025). For each case, the likelihood of detection under pre-PCR pathways was assessed. The Ct value for stx in the diagnostic sample was obtained. Strain characteristics, including serotype and stx subtype, were classified according to the UKHSA severity algorithms. Clinical and demographic variables were recorded. Severe disease was defined as: haemolytic uraemic syndrome, bloody diarrhoea, hospitalisation and/or death. Logistic regression examined associations between strain and patient characteristics with the odds of severe disease.

Findings
189 STEC cases were included. PCR increased STEC detection, with around half of cases unlikely to have been identified previously. 46% of all cases had severe disease. Although strain diversity was substantial, serotypes O157:H7, O103:H2, O26:H11 and O145:H28 were more often associated with severe disease. Infections with strain characteristics ranked severe by UKHSA had over fourfold increased odds of severe disease (OR 4.22, 95% CI 1.98–9.33). Although higher Ct values reduced culture confirmation rates, severe disease was observed in some culture-negative cases with moderate CT values.

Conclusions
Overall, the study offers insights that may help inform future approaches to STEC diagnosis, surveillance, and public health response.

Use of V-Quick32 Nucleic acid extraction system in streamlining molecular testing for STEC

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[1] NHS Lothian

Shiga toxin-producing E. coli (STEC) are foodborne pathogens which can cause severe systemic disease. At the Scottish E. coli O157/STEC Reference Laboratory (SERL), current faecal screening relies on manual DNA extraction which is time-consuming, labour intensive and requires streamlining due to the increased number of samples being submitted for screening.

Given the limited space available for equipment in our CL3 laboratory, the automated V-Quick32 system offers a small footprint option for nucleic acid extraction and is scalable up to 32 samples.

A panel (n=77) of known STEC positive and negative faecal samples, comprising a range of serotypes and stx subtypes, was processed using the V-Quick32 system followed by STEC detection using the Viasure E. coli EHEC, EPEC & EIEC Real Time (RT) PCR Detection Kit (VIASURE system). Results were compared with the current SERL method (enrichment, manual InstaGene extraction and in-house RT-PCR). Comparison with the current method gave a sensitivity of 96% and specificity of 100% for the detection of STEC using the V-Quick and Viasure device. Investigation of false negative results suggested that stx2i was not detected by the Viasure RT-PCR Detection Kit and one sample with very low levels of STEC DNA which was positive by in-house RT-PCR but negative using the VIASURE system.

The V-Quick32 system offers an efficient method of DNA extraction with the advantage of reduced hands-on processing time and potential to remove an overnight enrichment step.

Genetic variability of Shiga toxin-producing Escherichia coli (STEC) strains isolated from human cases of bloody diarrhea in Northern Italy

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Shiga toxin-producing (Stx) Escherichia coli (STEC) infection can cause different clinical symptoms from bloody diarrhea to hemolytic uremic syndrome (HUS), depending on the Stx produced and to the genetic properties of the strain involved. The main serogroups responsible of STEC infections are O157 and O26, which together with O103, O111, O145 are termed as "top-5". However, STEC strains belonging to other serogroups are often reported. This study aimed at characterizing, through genomic approach (WGS), STEC strains isolated from bloody diarrhea collected by North Italian pediatric hospitals from 2018 to 2025. WGS was performed on 151 STEC strains. Most of them harbored the stx2 gene alone (46%), which is associated to severe outcomes, in particular the variants stx2a (32%), and stx2c (7%). The combination of stx1+stx2 genes was found in 30% of strains, while 23% carried stx1 alone, mainly being stx1a (19%). The eae gene, coding for intimin, was identified in 89% of strains. Overall, 26 different serogroups were found, most of which belonged to the "top-5", especially O157 (33%), and O26 (25%). The main non-"top-5" serogroups were O71 (4%), which harbored stx1 alone (serotype O71:H2) or stx1+stx2 (serotype O71:H8), and O177 (3%), carrying only stx2 (serotype O177:H25). Although the most frequently detected STEC serogroups are O157 and O26, the presence of emergent serogroups, such as O177 and O71, underline the importance of monitoring the circulation of STEC. Our study also highlights the importance of STEC genetic characterization, due to their variability and capability to exchange genes coding for virulence determinants.

Peptide-based inhibitors targeting the ribosomal binding site of Shiga toxins

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Background

Shiga toxins exert their toxicity by enzymatically depurinating the conserved sarcin/ricin loop (SRL) of the large ribosomal subunit in host cells. The catalytic A1 subunit (StxA1) engages the C-terminal region of the ribosomal stalk P-protein to gain access to the SRL. Using X-ray crystallography and cryo-EM, we resolved structures of Stx2a and Stx2A1 bound either to a peptide mimicking the conserved P-protein C-terminus or to the purified P-protein complex, defining the molecular basis of this interaction.

Rationale

The ribosome-binding site is accessible on both the isolated StxA1 subunit and the intact holotoxin, making it a viable therapeutic target. Peptides that mimic the P-protein C-terminal sequence inhibit StxA1 activity, establishing this interface as a foundation for the development of antidotes. Targeting this site offers two strategic advantages. First, inhibitors can neutralize holotoxin before it enters the cell and disable the A1 subunit once it is internalized. Second, the binding site is conserved across all Shiga toxin variants, enabling broad-spectrum inhibition.

Finding

We have synthesized and evaluated modified peptidomimetics in vitro, identifying candidates with substantially improved binding affinity and inhibitory potency compared to native peptides.

Conclusion

These findings underscore the ribosome-binding interface as a promising, conserved target for peptide-based inhibitors to counteract Shiga toxins. Future work will prioritize enhancing the affinity, cellular uptake, and stability to advance of the lead compounds toward therapeutic application.

Shifting age distributions among STEC O157 and O26 infections over time in the Netherlands

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STEC O157 is notifiable in the Netherlands since 1999. The surveillance was extended to all STEC serotypes in 2007, when molecular methods for detection of STEC became available. Patient isolates are voluntarily submitted for genotyping.

Longterm trends in characteristics of cases with STEC-infections were examined. Between 2000 and 2025, a total of 10883 STEC-infections were reported. The serotype was available for 3627 reports of which 1471 STEC O157 and 355 STEC O26. Median age of all cases was 40 years (0-100 years), whereas the cases with STEC O157- and O26-infections were younger: 23 and 20 years, respectively. However, the age distribution shifted over the years. In 2000, the median age of STEC O157-cases was 17 years, increasing to 27 years in 2024. For STEC O26-infections, the median age fluctuated around 18 years in 2011-2021, but increased to 25-26.5 years (2022-2023) and 43 years (2024). Examining hospitalization and HUS show lower occurrences of HUS related to STEC O157 since 2009 (2-8%) compared to 2000-2008 (8-24%); a decrease in hospitalization is only seen in 2022-2024 (24-26%; 2000-2021: 27-54%). Hospitalization due to STEC O26 increased with 17-24% of cases in 2011-2016 compared to 32-54% (2017-2024). The number of HUS cases per year related to STEC O26 were to low for examination of trends.

STEC infections, especially O157 and O26, are seen as infections particularly affecting children, this increasing age is a trend that needs to be followed up.

Genetic diversity and temporal dynamics of stx2-carrying STEC strains in a French cattle farm

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Background
Shiga-toxin producing Escherichia coli (STEC) cause severe human diseases, notably hemolytic uremic syndrome (HUS). While French public health authorities historically targeted "Top 5" serotypes, current data emphasize the pathogenic potential of all stx2-carrying strains and the emergence of virulent hybrid clones like O80:H2, now a leading cause of HUS in France for which a reservoir has yet to be identified.

Rationale for the work
Cattle are a major reservoir of STEC, yet the epidemiology of non-O157 stx2-positive strains remains underexplored. Assessing the genomic diversity and long-term persistence of these strains within cattle farms is crucial to update risk assessments and control strategies beyond the traditional "Top 5".

Findings
We monitored a French cattle farm naturally infected by stx2-positive STEC strains over 14 months. Genomic characterization identified 14 serotypes, notably including the top three associated with pediatric HUS in France: O80:H2, O26:H11, and O157:H7. Seven clones could be considered as highly pathogenic (carrying the stx2a or stx2d subtypes of the stx2 gene, and sometimes the eae gene). A major finding was the identification of a hyper-virulent STEC-ExPEC O80:H2 clone that persisted throughout the study. This clone, genetically highly similar to French clinical strains, showed high shedding prevalence in healthy animals (up to 73.2%).

Conclusions
Our findings indicate that healthy cattle can act as a sustainable reservoir for highly pathogenic stx2-carrying STEC, including O80:H2. Enhanced surveillance both at farm and food-chain levels, coupled with genomic investigations of persistent clones, is essential to better anticipate transmission risks and guide targeted control strategies.

Insights into the diagnostic approach of STEC-HUS: registry and survey data from the European Rare Kidney Disease Reference Network

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Background and rationale
The European Rare Kidney Disease Reference Network (ERKNet), a consortium of 72 expert nephrology centers, has created the European Rare Kidney Disease Registry (ERKReg) to collect data on patients with rare kidney diseases, including STEC-induced hemolytic uremic syndrome (HUS). To assess current practice in the diagnosis of STEC-HUS, we performed a mixed-method study using ERKReg registry data and surveying ERKNet-affiliated centers on their diagnostic approach to STEC-HUS. Patients with STEC-HUS enrolled in the registry from January 2019 to January 2025 were included.

Findings
In total, 647 patients were included. STEC was detected in 516/573 (90%) patients. Shiga toxin PCR, stool culture, and serology identified STEC in 317/390 (81%), 384/505 (76%), and 120/185 (65%) patients, respectively. Serotype and Shiga toxin type were not reported. Of the 381 patients evaluated with at least two different diagnostic tests, 105 (28%) showed discordant results. For instance, STEC was PCR-negative but stool culture-positive in 24 patients.

The survey was completed by 36/72 (50%) centers. While almost all centers (n=35) reported performing fecal diagnostics, only 8 (22%) centers used serology. Notably, centers employed diverse PCR and stool culture methods and varied in the serotypes they could detect. In 28/35 (80%) centers, rectal swaps were used if stool collection was not possible. Collection of multiple stool samples is performed in 14/35 (40%) centers.

Conclusions
Currently, there is considerable variation in the diagnostic approach to STEC as a cause of HUS. Developing a practical guideline for STEC diagnosis and serotyping is recommended.

Detection of Enteroinvasive Escherichia coli (EIEC) outbreak in Alberta, Canada

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Background:
In July 2025, a gastrointestinal outbreak of EIEC along with other enteric pathogens was reported in a restaurant in Alberta. Initial test results showed positive detection of the Shigella/EIEC target by the BD MaxTM Enteric Bacterial Panel PCR at frontline diagnostic laboratories and the stool culture confirmed the pathogen as an ESBL-producing EIEC. To identify and distinguish Shigella from EIEC on all subsequent stools submitted to Alberta Precision Laboratories-Alberta Public Health, a stepwise testing algorithm was developed.

Methods:
Stools positive on the BD Max for Shigella/EIEC were submitted to ProvLab for isolation and were cultured onto a new Shigella CHROMagar; red colonies, indicative of non-Shigella spp. were sub-cultured onto CHROMagar™ ESBL agar. From each patient, 1-3 colony picks were screened by PCR targeting ipaH which was validated for sensitivity and specificity prior to use. One positive isolate/patient was used for whole genome sequencing (WGS).

Results:
ipaH PCR was specific for Shigella/EIEC with a detection level at 700 CFU/mL. Stools (n=101) were cultured and ipaH PCR performed on 259 colonies. In total, 31 isolates (31%) were recovered and serotyped by WGS as O96:H19. Based on wgMLST cluster analysis, these isolates were highly clonal (<7 alleles apart) and clustered with 2 other outbreak-related EIEC isolates confirmed by WGS after presumptive identification by BD Max, Vitek GNI and MALDI.

Conclusion:
EIEC is not a notifiable disease in Alberta and this is the first EIEC outbreak reported. By stepwise culture and PCR, EIEC O96:H19 was identified as a contributing pathogen of this outbreak.

Shiga toxin-producing Escherichia coli are not associated with bloody diarrhoea in Ibadan, Nigeria

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Shiga toxin-producing Escherichia coli (STEC) reports from West Africa are few, perhaps due to low incidence or to difficulties in laboratory detection. Accessible diagnostic protocols seek O157 or enterohaemorrhagic strains but can miss 'atypical' and hybrid STEC. We screened stools specimens from diarrhoea in people living with HIV (PLWHIV) and HIV uninfected controls for STEC using molecular methods. Patients with diarrhoea – PLWHIV (n=127) and HIV-uninfected (n=202) attending two Ibadan hospitals between January 2019 and February 2021 were recruited. Their stool specimens were screened for occult blood via immunochemical testing and cultured on MacConkey and eosin methylene blue agars. Colonies were screened by PCR for stx1, stx2, eae and virulence genes of other diarrhoeagenic E. coli. All stx-positive and stx-negative isolates, by PCR, were Illumina sequenced. VirulenceFinder identified virulence genes while multilocus sequence typing and EcTyper delineated sequence types and serotypes, respectively, from quality assured genomes. Data were analysed using Chi square and Fisher's Exact tests, at α0.05.

Forty-nine (38.6%) stool specimens from PLWHIV and 31(15.3%) from HIV-uninfected contained detectable blood (p=0.000003). stx1 (10/264, 3.9%) and stx2 (3/264, 1.1%) were uncommonly detected by PCR with 2 (15.4%) PCR-positives cultured from bloody stools. Upon sequencing, stx genes were not found in genomes of any PCR-positive isolate or in 467 PCR-negative isolates sequenced. Only one PCR-positive isolate belonged to a serovar or sequence type ever associated with STEC.
While bloody diarrhoea is common among PLWHIV, STEC are rare and are unassociated with bloody diarrhoea in Ibadan. Nonetheless, continued STEC surveillance is encouraged.

Temporal Pangenome Dynamics and Functional Diversity of Escherichia coli O26 from a Cattle Farm

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Background:
Escherichia coli O26 is a clinically significant zoonotic lineage within Shiga toxin–producing E. coli, with cattle as its primary reservoir. Understanding its genomic diversity and evolution within farm environments is essential for assessing persistence and zoonotic risks. Rationale: Longitudinal genomic datasets for E. coli O26 remain scarce, limiting insight into within-farm population dynamics. To characterise temporal structure, accessory genome flux and functional diversity, we analysed 20 E. coli O26:H11 isolates collected across four farm visits over one year, representing STEC O26 ST21(n=12) and eae-positive non-STEC O26(n=8) ST29.

Main Findings:
The pangenome comprised 4,519 core genes, 1,402 accessory genes and 17 unique genes, indicating a near-closed pangenome. Diversity metrics (Shannon ≈12.34) and Jaccard similarity clustering showed moderate diversity. ST21 persisted across all visits, gaining most accessory genes between visits 1–3 and loses many genes by visit 4. ST29 emerged at visit 3 and showed minimal change by Visit 4. Functional categorisation of the pangenome showed dominant Clusters of Orthologous Genes (COG) classes for transcription, ion transport, energy metabolism, and cell envelope biogenesis, with many genes of unknown function. The PFAMS (protein families database) and KEGG (Kyoto encyclopedia of genes and genomes) profiles supported these patterns, highlighting differences in transport, regulatory, and metabolic features between ST21 and ST29.

Conclusion:
These findings provide insight into the dynamic composition of the E. coli O26 genome, by revealing lineage-specific functional and temporal variations in the accessory genome of isolates collected from the same farm, indicating continual genomic evolution enables niche-adaptation and persistence on farm.

Evaluation of Soluplus®–lactoferrin nanomicelles against STEC: in vivo biodistribution and effects in an infant's colon model

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Bovine lactoferrin (bLF) is a glycoprotein with antibacterial activity. We previously developed Soluplus® nanomicelles associated with bLF (NM–bLF), which increased bLF properties against Shiga toxin-producing Escherichia coli (STEC) in vitro. Here, we analyzed the biodistribution of NM-bLF using the in vivo imaging system (IVIS) and its effect against STEC using the ARtificial COLon (ARCOL). For biodistribution, fluorescent bLF or NM–bLF were administered intragastrically to adult BALB/c mice, and ventral, dorsal, and fecal fluorescence were registered periodically. To analyze the antibacterial effect, 1x10^7 CFU/ml of STEC were added to the ARCOL containing a toddler's healthy fecal sample. Then, treatments (NM-bLF 0.1%-1mg/ml, bLF 1mg/ml, NM 0.1%) were administered daily for three days. STEC (CFU/ml) was detected on SMAC agar, expression of stx2a, eae, and lpfA was assessed by RT-qPCR, and Stx2a cytotoxicity was assessed on Vero cells. Similar biodistribution for bLF and NM-bLF was observed in the digestive system and fecal fluorescence was detected at 3h in both conditions. Furthermore, NM-bLF and bLF reduced similarly CFU/mL of STEC in the ARCOL after 24h of treatment compared to NM alone (*p<0.05). NM-bLF and bLF upregulated the gene expression of stx2a, eae, and lpfA at 12, 24, and 36h compared to NM. However, NM-bLF showed lower expression of those genes 24h after treatment (*p<0.05) and reduced cell death due to Stx2 on Vero cells compared to bLF (****p<0.0001). These results suggest that NM may be proposed as a safety nanoplatform for the delivery and enhancement of therapeutic compounds against STEC.

Occurrence and characterization of Shiga toxin-producing Escherichia coli from wild ruminants in North-west Italy

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Background
Shiga toxin-producing Escherichia coli (STEC) are an important cause of disease in humans. Ruminants, particularly cattle, are recognized as STEC natural reservoir. Beside domestic ruminants, wild ruminants may also act as a reservoir.

Rationale for the work. 563 livers of hunted wild ruminants collected in Liguria, north-west Italy, from 2019 to 2025, and 34 samples collected in Aosta Valley in 2023-2024 were examined. Liver samples (25 g) were weighted and homogenized in 225 ml of buffered peptone water. A protocol slightly modified from the ISO TS 13136:2012 was used for STEC detection and isolation. The isolated STEC strains are currently being typed in terms of serotype, ST, stx subtype and virulotype by whole genome sequencing (WGS), to characterize STEC circulating in wildlife in the study area.

Main findings
Forty samples were found positive to stx1 and/or stx 2 in Liguria (percentage of positivity 7.1%), and 5 in Aosta Valley (14.7%), and 72 colonies positive for stx1 and/or stx2 were isolated from these 45 samples. Preliminary WGS results indicate that they belong to different serogroups and display several stx subtypes, including stx1c, stx1b, stx2b, stx2e and stx2g. Some strains possess subAB genes, described also in LEE negative STEC strains isolated from human cases of disease. Interestingly, the strains possessing stx2g display cross-pathotype characteristics, harbouring the sta1 gene, which encodes the heat sable enterotoxin described in Enterotoxigenic E. coli (ETEC).

Conclusions
This study highlights the importance of wildlife monitoring in a "One Health" approach.

Shiga toxin delivered in extracellular vesicles induces higher mortality and more kidney injury than free toxin

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Escherichia coli O157:H7 is a non-invasive Shiga toxin (Stx)-producing pathogen that causes gastroenteritis and hemolytic uremic syndrome characterized by acute kidney injury. Stx binding to cells induces release of extracellular vesicles (EVs) containing the toxin.

This study aimed to demonstrate the lethal effects of toxin-positive vesicles compared to free toxin, and the importance of the Stx receptor, globotriaosylceramide (Gb3).

BALB/c mice were injected intravenously with Stx2 126 ng/kg, the same toxin concentration in Stx2-EVs, or toxin-negative EVs. Mortality was significantly increased in mice challenged with Stx2-EVs (n=8/8) compared to mice challenged with free Stx2 (n=4/7). Urea levels were significantly higher, and kidney tubulointerstitial pathology and fibrinogen staining more prominent in mice challenged with Stx2-EVs. Mice challenged with EVs alone (n=7) remained unaffected. C57BL/6 mice and Gb3-negative littermates were injected with Stx2-EVs at toxin concentrations of 126 or 200 ng/kg. Gb3-negative mice (n=7) injected with Stx2-EVs at both toxin concentrations were totally protected, whereas wild-type mice developed disease, n=2/4 at the lower and 4/5 at the higher toxin concentration. These mice remained unaffected by the same concentrations of free toxin (n=10).

This study shows that Stx2 delivered systemically within EVs is more lethal than free Stx2, causing more kidney damage, and the Gb3 toxin receptor is crucial for disease induction by circulating Stx2-EVs.

What is out there? – STEC in the Zoonosis-Monitoring in Germany

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STEC are food-borne zoonotic pathogens naturally occurring in farmed and wild ruminants. They are transmitted by contaminated food like raw milk and meat, but also plant-based food is reported regularly as infection vehicle. Clinical symptoms range from (bloody) diarrhea to HUS. Especially small children are at risk for severe disease.

Data on STEC in the food-chain are collected annually within the National Zoonosis Monitoring including matrices chosen on a risk-based decision.

We present data on STEC prevalence in Germany from 2012 to 2024 from a diverse range of matrices like roe deer (N=528; STEC prevalence 40%), game meat (N=978; STEC 16-31%), meat from beef cattle and veal (N=3383; STEC 0.9-6.7%), wheat flour (N=242; STEC 9%) or leafy greens (N=2171; STEC 0-2.2%). Additionally, typing data as stx-subtype and serotype are discussed for specific matrices. STEC from roe deer and meat thereof were dominated by serotype O146:H28 encoding stx2b. Beef had a high proportion of STEC O113:H4/H21 with stx2a/stx2d. Wheat flour at mill was contaminated with O187:H28 containing stx2g. STEC from leafy greens showed a variety of serotypes and stx-subtypes including stx2f. STEC with stx2 and eae were found in 7% (N=117) of the analyzed STEC isolates (N=1651), and were mostly from animal faeces (82%) but also from plant-based food (5%).

STEC prevalence data are publicly available by the online tool ZooNotify (**https://zoonotify.bfr.berlin/?locale=en**), and can be used also for risk assessment. Whole genome sequence data are increasingly integrated and will enhance the monitoring of zoonotic pathogens and antimicrobial resistance.

Assessing Performance of a Method of Analysis for Shiga toxin producing Escherichia coli in food

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Analysis of food for Shiga toxin producing Escherichia coli (STEC) is challenging due to STEC diversity, and the diverse foods they are found in. To improve STEC detection for Canadian food safety programs, a method of analysis was developed that features: improved enrichment of injured cells; robust DNA extraction; real-time PCR for the Shiga toxin gene in enrichment media and colonies; colony isolation in parallel on low selectivity and high selectivity agar media; and real-time PCR for colonisation factors (eae, aggR and aaiC) associated with severe illness.

The performance of this method was assessed to the requirements of the Health Canada Compendium of Analytical Methods. The method is inclusive of diverse STEC and detects 19 Stx-subtypes. The limit of detection with isolation approaches 1 cell in 325 g of ground beef, 1 cell in 100 g of Romaine lettuce or raw milk cheese. Heat injured STEC were isolated from 90% of ground beef samples and 75% of lettuce samples. Lactic acid injured STEC were isolated from 95% of raw milk cheese samples. Performance analysis with 25 g analytical units was assessed with eighteen food items from Dairy, Fresh Fruits and Vegetables, and Raw Meats categories. Enrichment screening performance was Sensitivity 99.6%; Specificity 100%; False Negative Rate 0.4%; False Positive Rate 0%; Efficacy 99.8%. STEC isolation performance was Sensitivity 98.5%; Specificity 100%; False Negative Rate 1.5%; False Positive Rate 0%; Efficacy 99.1%.

This method offers significant improvements over the established Canadian STEC method and is being implemented into Canadian testing programs.

Predicting STEC culture positivity rates based on PCR Ct values

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Background
PCR assays targeting the Shiga toxin gene (stx) provide a robust, specific and sensitive diagnostic test for the detection of STEC. However, clinical and public health management of individual cases and outbreaks requires characterisation of cultured isolates. Due to the lack of selective agar, culture of Shiga toxin producing Escherichia coli (STEC) other than O157, is labour intensive and expensive.

Rationale for the work: PCR ct values provide an indication of STEC bacterial load within a faecal specimen (the lower the ct value, the higher the bacterial load). We reviewed PCR ct values to determine whether there was a cut off after which obtaining a positive culture would be unlikely, enabling resources to be focused on specimens most likely to be culture positive.

Main findings or outcomes
There were 485 faecal specimens included in the study, divided into three groups with ct value <30 (n= 177), 30-35 (n= 277) and >35 (n=81). Of these, 296/485 (61.0%) were culture positive. As expected, the highest proportion of culture positive specimens had ct values <30 (<30 n=143/177, 80.8%; 30-35 n=121/277, 53.3%; >35 n=32/81, 39.5%).

Conclusions
Culture was twice as likely to be successful in specimens where the ct value was <30 compared to >35. However, culture was possible where the bacterial load was low. Although ct value can be used to predict successful culture, we recommend specimens testing positive for stx from hospitalised patients, children aged <5 years old and community cases reporting bloody diarrhoea should be cultured regardless of ct value.

Targeting EHEC Virulence: Aurodox Inhibits Type III Secretion Independently of PurA

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Therapeutic options for Shiga toxin–producing Escherichia coli (STEC) remain limited, motivating interest in anti-virulence strategies that suppress pathogenicity without inducing Shiga toxin expression. Aurodox, originally characterised as an antibiotic, is a potent inhibitor of the type III secretion system (T3SS) at sub-inhibitory concentrations and confers protection in a murine STEC infection model. Despite this, its molecular target remains unclear. Previous work in enteropathogenic E. coli (EPEC) identified adenylosuccinate synthase (PurA) as the primary target of aurodox.

Here, we investigated whether PurA is the molecular target for aurodox-mediated T3SS inhibition in the Shiga toxin-negative EHEC laboratory strain TUV93-0. We compared wild-type and ΔpurA TUV93-0 strains using mammalian cell adhesion assays, transcriptomic analysis, protein secretion assays, and LEE reporter assays, in the presence and absence of aurodox. When adenine supplementation was provided to rescue the auxotrophy of the ΔpurA mutant, aurodox retained full inhibitory activity against the T3SS, with no detectable difference compared to wild-type.

These data do not support purA as the principal target of aurodox in this EHEC model and instead suggest that aurodox may act through alternative or strain-specific targets. Ongoing work aims to identify the direct molecular target(s) of aurodox in EHEC, providing mechanistic insight to support its development as an anti-virulence therapeutic.

Validation of Novaplex stx1/2/2a/2d PCR for Identifying High-Risk Shiga Toxin 2 Subtypes in Clinical Samples

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Background
Among the Shiga toxin–producing E. coli (STEC), subtypes Stx2a, Stx2c, and Stx2d are classified as highly virulent in some countries, including Norway, while other countries do not classify Stx2c as highly virulent.

Rationale for the work
When the most virulent Stx2 subtypes are detected, specific infection control measures are recommended. Because such measures can impose socioeconomic burdens, rapid and accurate Stx2 subtyping is essential for risk assessment and surveillance.

Methods
This study evaluated the Novaplex™ stx1/2/2a/2d Typing (RUO) kit (Seegene, Korea), a multiplex real-time PCR assay designed to detect stx1, stx2, and specifically subtype stx2a and stx2d, on a panel of 128 STEC strains, including reference strains and whole genome sequenced clinical strains, representing eight different Stx2 subtypes.

Results
All isolates were classified as Stx2 (n=128). Subtypes Stx2a (n=53) and Stx2d (n=4) were accurately identified, whereas Stx2l (n=1) was misclassified as Stx2a and Stx2d. No misclassification of other subtypes (Stx2b/c/e/f/g/i) into Stx2a or Stx2d was observed.

Conclusions
The Seegene Novaplex assay demonstrates reliable performance for subtyping Stx2a and Stx2d, with few false positives. Distinguishing Stx2l from Stx2a and Stx2d remains challenging due to the high genetic sequence similarity among these variants, particularly in regions commonly targeted by PCR-based detection methods. Notably, the assay is not designed to detect stx2c. While the method may support the continuation of infection control measures, it cannot be relied upon to guide de-escalation and is therefore not yet suitable for routine diagnostic use in countries where Stx2c is classified as highly virulent.

Enterohaemorrhagic Escherichia coli Shiga Toxins Promote Necroptosis via Mitochondrial DNA-Mediated cGAS-STING Activation in Monocytic cells.

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Background:
Shiga toxins (Stxs), the primary virulence factors of Enterohaemorrhagic Escherichia coli (EHEC), may contribute to HUS-associated renal injury through apoptosis.

Rationale for the work:
Although Stx-induced apoptosis has been extensively characterized as a form of programmed cell death, treatment with the pan-caspase inhibitor Z-VAD-fmk failed to fully inhibit cell death, suggesting the involvement of a caspase-independent mechanism.

Main findings or outcomes:
In this study, we used the Stx-sensitive monocytic THP-1 cell line to investigate the involvement of necroptosis. Exposure to Stx2a induced ROS-dependent mitochondrial damage, leading to cytosolic mtDNA leakage and subsequently activating the cGAS-STING pathway, promoting TNF-α-mediated necroptosis. Consistently, we detected phosphorylation of RIPK1, RIPK3 and MLKL, hallmarks of necroptosis. Pharmacological inhibition using Necrostatin-5 (Nec-5), GSK-872 and Necrosulfonamide (NSA) confirmed the functional involvement of the necroptosis machinery.

Conclusion:
Together, these findings demonstrate that Stx2a-induced ROS promotes mtDNA release and triggers cGAS-STING-mediated necroptosis through TNF-α signaling. This study provides new mechanistic insights into Stx-induced host cell death pathways and highlights an underexplored axis of bacterial toxin-mediated immune pathology.

Genetic characterization of Shiga-toxin-producing Escherichia coli (STEC) isolated from raw milk cheese in northern Italy

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Shiga-toxin-producing Escherichia coli (STEC) cause symptoms ranging from diarrhea to haemolytic uremic syndrome (HUS), depending on the Shiga toxin produced (Stx1, and Stx2), with serious consequences in terms of human lives and healthcare costs. Cattle are their natural reservoir, and the ingestion of contaminated dairy products, particularly raw milk cheeses, are associated with STEC infections. In Italy, raw milk cheese consumption is traditional and popular. This study aimed to characterize, through genomic approach, STEC strains isolated from raw milk cheeses from 2018 to 2025, providing an overview of their genetic characteristics and diversity.

Overall, 70 STEC strains were isolated from different types of raw milk cheeses in Northern Italy. Most of the strains carried stx1 gene (66%), whereas the combination of genes associated with severe symptoms (stx2 and eae, which codes for intimin) was found in 34% of the isolates. There was a variety of 29 serogroups, including those involved in paediatric HUS and outbreaks (O26, and O103), and emerging serogroups (O80, O113, and O128), highlighting the importance of monitoring the circulating strains to assess their impact on human health. Overall, 67% of the strains carried AMR genes, conferring resistance to β-lactams (61.4%), streptomycin and kanamycin (18.5%), and tetracycline (14.3%). The phylogenetic analysis confirmed the circulation of diverse strains in dairy production environments.

The consumption of raw milk cheeses represents a risk for STEC infection, especially in young children and vulnerable individuals, highlighting the importance of a correct consumer information and education to prevent STEC infections and improve food safety.

Cellular signaling pathway of Shiga toxin-induced ATP release

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Background:
Shiga toxin (Stx) is the main virulence factor of enterohemorrhagic Escherichia coli, a food-borne pathogen that colonizes the intestine causing gastroenteritis and, in severe cases, hemolytic uremic syndrome. Stx was shown to induce ATP release in vivo and in vitro and blockade of purinergic P2X receptors inhibited its cytotoxicity. Here we investigated the intracellular signaling events preceding ATP release.

Methods:
Inhibitors included pertussis toxin, wortmannin, manoalide, 2-aminoethoxydiphenylborate (2-APB), BAPTA-AM and Ca2+-free medium. The inositol 1,4,5-triphosphate receptor (IP3R) was silenced. Stx-induced apoptosis was detected by caspase 3/7 activation. BALB/c mice were injected with Stx2 i.p. Certain mice were pretreated with alpelisib (1 h before and 24 h after Stx2). Kidneys collected after 4 days were stained for phosphatidylinositol 4,5-bisphosphate (PIP2).

Results:
Stx1-mediated ATP release in HeLa cells was blocked by pertussis toxin affecting the Gi/o family of G-protein coupled receptors. ATP release was also abrogated by wortmannin, an inhibitor of phosphoinositide 3-kinase (PI3K), by manoalide, inhibiting phospholipase C, by 2-APB inhibiting IP3R, and by reduction of intracellular calcium (BAPTA-AM) and extracellular calcium (Ca2+-free medium). Blocking or silencing the IP3R protected HeLa cells from Stx1-induced apoptosis. Likewise, blocking the IP3R reduced Stx2-induced apoptosis. Stx2-challenged mice had distinct PIP2 glomerular staining that decreased in the presence of the PI3K inhibitor alpelisib.

Conclusion:
Stx interaction with HeLa cells initiates a signaling pathway involving membrane G protein, PI3K, phospholipase C and IP3R, ultimately leading to ATP release and promoting cytotoxic effects. The PI3K inhibitor alpelisib altered PIP2 expression in Stx2-challenged mice.

An investigation of Shiga-toxin producing E. coli (STEC) distribution and diversity within a typical Scottish dairy herd

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The STEC reservoir posed by Scottish cattle intended for slaughter has been extensively studied. Little is known about the Scottish dairy sector.

As a pilot, before embarking on a wider study of the diversity of STEC in the Scottish dairy cattle sector, we collected 186 faecal pat samples from six management groups in one Scottish dairy herd, in 2023.

A single time point sampling protocol was used. We took sufficient samples to be 90% certain that we can detect STEC O157, if one or more animals in the group are shedding it.

Using culture techniques with PCR confirmation of the presence of O157, eae, stx1 and stx2 genes, O157 STEC were obtained from only one management group, the milking herd. All seven isolates screened positive for stx1+2 and eae on PCR.

The within-herd frequency of non-O157 STEC-positive pat samples was high. Non-O157 STEC-positive samples occurred most frequently in the two youngest management groups (youngstock 2-13 months old and pre-weaned calves). These groups were also the most diverse, with all three stx types (stx1, stx2, stx1+2) found in both.

The apparent greater diversity of non-O157 STEC stx types within these groups may reflect the more dynamic management of younger cattle, with new animals entering the groups relatively frequently, whereas the older management groups will be more stable. All isolates have been sequenced; we await outputs from the analysis of the genome sequence data to further this investigation.

Asymptomatic Shiga toxin-producing Escherichia coli (STEC) carriers among people working or living on livestock farms in the Netherlands.

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Background and Rationale for the work
To obtain data on the occurrence and trends of zoonotic agents in humans and animals (Zoonoses Directive (2003/99/EC) the Netherlands has implemented from 2013 onwards a surveillance program. This program monitors the prevalence of various potential pathogenic bacteria, including STEC, in livestock farm animals and in humans working or living on these farms. The following sectors have been investigated; beef cattle, broilers, dairy cattle, dairy goats & sheep, layers, meat sheep, pigs, and veal calves.

Main findings or outcomes
Of the 666 humans who participated, 24 were found stx PCR positive and for 22 a STEC isolate was successfully isolated from the stool sample. The prevalence among these participants differed between the livestock sectors investigated, with the highest among the dairy goats & sheep (4.9% (8/162)) and meat sheep (11.1% (9/81)) sectors.

STEC prevalence in the animals varied from 21.4% to 99.5% at farm level, with the highest percentages on dairy goats & sheep farms.

After WGS analysis, fifteen different STEC serotypes were identified among the 22 human isolates, with O146:H21 (n=7) being the most common one. Seven different stx gene profiles were determined with stx1c & stx2b (n=7), stx1a (n=5), and stx1c (n=5) most frequently found.

Five of the human isolates clustered (1-20 AD with cgMLST analysis) together with an animal STEC from the same farm.

Conclusions
Asymptomatic STEC carriers were found among 3.3% of the participants, mainly from dairy goats & sheep and meat sheep farms.

Management of STEC cases in Norway – revision of national guidelines

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Background
In Norway, STEC cases have increased from 2013, with a 2024 notification rate of 11.8 per 100,000 reported to the Norwegian Surveillance System for Communicable Disease (MSIS). Since 2016, interventions have focused on high virulent STEC, with strict guidelines requiring three negative fecal samples for clearance.

Rationale for the work
To ensure minimal retention from work or kindergarten, we reviewed the duration of pathogen shedding post-STEC infection, the number of negative fecal samples needed, and the methods used for confirming microbiological clearance. This involved a systematic literature search (2004-2024), analysis of all laboratory results from Norwegian microbiological laboratories available in MSIS-laboratory database (MSIS-lab, 2023-2024) and mapping national guidelines on STEC management from various countries.

Main findings
The literature search identified 2309 studies, with 14 meeting the inclusion criteria. Median shedding ranged from 10-32 days, with a few studies reported prolonged shedding in younger children. Clearance required 1-5 consecutive negative fecal samples, confirmed by culture (8 studies), PCR (4 studies), or both (2 studies). MSIS-lab data indicated a median shedding of 14 days, consistent across ages. Among cases with three follow-up samples, 86% had three consecutive negative results. In four cases, two negative samples were followed by a positive, with PCR alone identifying positives in two cases. Notably, 90% of national guidelines recommended two negative fecal samples confirmed by PCR and/or culture.

Conclusions
Norwegian guidelines will be revised to require two consecutive culture-negative fecal samples for high virulent STEC cases in high-risk groups, aligning with guidelines from other countries.

Genomic characterization of STEC isolates from oral fluids of grower pigs and accompanying metagenomic insights

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Edema disease in pigs, a frequently fatal enterotoxemia caused by Shiga toxin–producing Escherichia coli (STEC), typically strains carrying F18 fimbriae and the stx2e gene. This study aimed to conduct herd-level surveillance and early detection of circulating stx2e-STEC strains in grower pigs using non-invasive oral fluid sampling.

Pen-level oral fluids (n = 58) were collected from growers aged five to 14 weeks on 15 Slovenian farms. STEC isolates were obtained according to ISO/TS 13136:2012 and characterized by whole-genome sequencing (WGS). To assess within-pen diversity, up to six STEC isolates per sample were further analyzed. Additionally, total DNA from five oral fluid samples was extracted using the NucleoSpin VET kit and subjected to Illumina shotgun metagenomic sequencing.

In total, 36 STEC isolates were recovered from eight pig farms. All isolates were stx2e-positive and belonged to seven sequence types and nine serotypes. The cgMLST analysis revealed that clonal isolates were mostly limited to a single farm, and multiple strains were found on four farms. Hybrid ETEC/STEC strains were detected on three farms; all harbored the heat-stable enterotoxin gene stb, and on one of these farms, sta1-positive isolates were also identified.

Metagenomic analysis of five samples showed that stx2 detection was consistent with the ISO STEC isolation method, although read mapping alone was insufficient to resolve the stx2 subtype.

Preliminary data show substantial between-farm genetic diversity of stx2e-STEC strains in growers and support the use of oral fluid as a suitable matrix for herd-level STEC monitoring using the ISO method and/or metagenomic sequencing.

Recent emergence of Shiga toxin-producing Escherichia coli serotype O171 among cattle in Japan

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Ruminants are a major reservoir of Shiga-toxin producing Escherichia coli (STEC) and carry various O-serogroups of STEC in their intestinal tract. In Japan, the serotype prevalence of the STEC isolated from bovine fecal samples was investigated in 1998, 2007, and 2013, which revealed the serotype shift among bovine STEC in several years. However, no update on bovine STEC isolates was reported in recent years.
To reveal the recent trend of STEC serotypes among cattle in Japan, we collected bovine fecal samples at a slaughterhouse from 2022 to 2024 and isolated STEC from them. We investigated 236 fecal samples in total and recovered 83 isolates from 76 samples. We identified 26 different O-serotypes/-genotypes, although most of them were isolated only once or twice during the investigation period. Intriguingly, 49 of 83 isolates (59%) were classified as O171, which were isolated from at least 24 different farms. We then sequenced the whole genomes of the isolates and performed phylogenetic analysis with the genomes downloaded from a public database. STEC O171 isolated from 2022 to 2024 formed two major clades, which were separated from STEC O171 isolated outside of Japan or STEC O171 isolated between 2013 and 2014 in Japan. One human isolate from an asymptomatic carrier was also clustered with the bovine STEC O171 clade. Phylodynamic analysis using BEAST2 implied that two major clades of O171 emerged almost within a decade in Japan. These data suggest the recent spread of clonal STEC O171 isolates among cattle in Japan.

Possible prolonged STEC shedding in the context of underlying gut pathology

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Background:
This case report describes an extended episode of Shiga toxin-producing Escherichia coli (STEC) infection with complex clinical and public health implications.

Case Timeline:
The patient tested positive for STEC from October 2021 to February 2022.
Two negative clearance samples were obtained. The individual then became symptomatic again in October 2022, with testing confirming non-O157 STEC (O128:H2, stx2b, sequence type 25). Whole-genome sequencing revealed identical typing between isolates, with cgMLST and SNP analysis indicating near-identical strains, suggesting persistence or possible reinfection from the same source. No clear exposure was identified. Significant gastrointestinal symptoms and iron deficiency persisted into early 2023, prompting referral to surgery and infectious diseases. Subsequent colonoscopy revealed multiple adenomatous polyps and bowel inflammation.

Public Health Measures:
The individual was in a risk group due to their occupation, requiring use of the Public Health Act to restrict duties. This had significant financial and wellbeing implications for the individual. Proportionate management of risk was agreed through ongoing multidisciplinary input.

Discussion:
Without genomic sequencing, it would not have been possible to determine that these infections were linked by an identical strain. Discussions with the Scottish E. coli Reference Laboratory raised the possibility of intermittent shedding due to uneven organism distribution and low-level excretion linked to underlying gut pathology.

Conclusion:
This case highlights the challenges of managing prolonged STEC carriage. It also highlights the key role that genomic analysis can play in epidemiological investigation. Crucially, it presents an important example of coordinated public health and clinical investigation and management.

The first cases of enteroaggregative Shiga toxin–producing Escherichia coli O104:H4 detected in Slovenia

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Background
Shiga toxin–producing Escherichia coli (STEC) can cause illness ranging from mild diarrhea to life-threatening hemolytic–uremic syndrome (HUS). Hybrid diarrheagenic E. coli strains that combine virulence traits of different pathotypes have emerged worldwide and represent a public health concern. One such hybrid, STEC-EAEC O104:H4, caused a large outbreak in Germany in 2011 linked to fenugreek sprouts. In 2023 and 2024, the first O104:H4 infections were identified in Slovenia.

Rationale
In Slovenia, specific genetic markers are used to routinely determine diarrheagenic E. coli (DEC) pathotypes, including STEC and enteroaggregative E. coli (EAEC). Whole-genome sequencing (WGS) is performed for all STEC isolates. We describe the first confirmed Slovenian cases of STEC-EAEC O104:H4: a 63-year-old man with bloody diarrhea after travel along the Adriatic coast (2023) and an 8-year-old child who developed HUS and acute kidney failure without recent travel history (2024).

Main findings
Both isolates belonged to sequence type ST678, carried stx2a, lacked eae, and contained EAEC-associated genes (aatA, aggR, aaiC). Additional important virulence genes (pic, sepA, sigA, lpfA) were identified. Both isolates harbored resistance genes blaTEM-1B, sul1, and dfrA7, but lacked blaCTX-M-15. A gyrA mutation (S83A) was detected in both strains. cgMLST showed the isolates clustered closely, differing by more than twenty alleles compared with the German outbreak strains.

Conclusions
These findings document the first detection of STEC-EAEC O104:H4 in Slovenia and suggest that hybrid pathotypes persist in Europe. Continued genomic characterization of diarrheagenic E. coli will support future investigations and strengthen public health preparedness.

Analysis of hamburgers during an outbreak of STEC O26 in Norway

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Background
In July to October 2023, Norway experienced one of its largest STEC outbreaks. Interviews pointed towards hamburgers from a domestic producer manufactured in a specific time period.

Rationale for work
Samples of frozen hamburgers, collected at retail, from patients' homes and from the producer, and raw material used for burger production were analysed using ISO TS 13136:2012. Samples positive for stx2, eae and O26 in the initial qPCR screening were selected for isolation. Presumptive STEC O26 (stx2 and eae positive) were submitted for WGS.

Main finding
STEC O26 (stx2, eae positive) were isolated from two samples of frozen hamburgers from patients' homes and two unopened packages from two different batches (A and B) collected from the producer. Seven isolates were sequenced and clustered with the outbreak strain using cgMLST. During analysis, one of 10 samples returned Cq values between 20 – 22 for the specific markers from batch A. After isolation, three of five pools were positive, and six of 30 colonies tested positive for all three markers. From batch B, nine of 17 samples were positive with variable Cq values for the selected markers. The four samples with lowest Cq values were selected for isolation. Only one pool from one sample was positive for all three markers and one colony was positive for stx2, eae and O26.

Conclusion
The results underline the importance of analysing more than one sample per batch due to low and heterogeneous contamination of a product as indicated in the products analysed here.

A Unitig-Based Genome-Wide Association Analysis of autoaggregation in enteroaggregative Escherichia coli

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Background:
Enteroaggregative Escherichia coli (EAEC) are important diarrhoeal pathogens and contributors to the evolution of Shiga toxin-producing E. coli (STEC) hybrids with exceptional colonization ability. Autoaggregation promotes biofilm formation and host colonisation but EAEC vary in their autoaggregation abilities and mechanisms. To identify significant factors in EAEC autoaggregation, which could potentially apply to hybrids, we performed a unitig-based genome-wide association study (GWAS) capable of detecting complex genomic variants.

Methods:
A unitig-based GWAS for autoaggregation (binary and continuous) was conducted on 380 EAEC genomes using pyseer's fixed-effect and mixed models, with 11 principal components included to account for population structure. The isolates were tested for autoaggregation by sedimentation assay.

Results:
Unitigs in colanic acid capsule biosynthesis and export genes were associated with autoaggregation. Pro-aggregation variants included cds-WP_001167296.1 (β=1.05), wcaJ (β=0.65), and wcaM (β=0.81). Anti-aggregation variants were detected in the wza-WP_162829205.1 intergenic region (β=-1.85 to -2.02), wzc (β=-1.79), wcaB (β=-1.85 to -2.04), cpsG (β=-1.79 to -1.90), yeaR (β=-2.52), and wcaA (β=-1.57). Notably, two unitigs spanning wcaC-wcaB had opposing effects: one was protective (β=-0.62 to -1.82) and the other a risk factor (β=+0.65). These unitigs also strongly predicted serotypes. The protective wcaC-wcaB unitig marked the H30 lineage that has been reported among STEC hybrids (OR=55.7) and O153:H30 subclone (OR=16.4), while the risk-factor unitig was associated with non-H30 strains (OR=0.060). The pro-aggregation cds-WP_001167296.1 variant marked O153 (OR=131.1) and O153:H30 (OR=226.2), and the anti-aggregation wzc variant defined H30 (OR=60.7).

Conclusion:
Specific capsule locus alleles, which are also serotype markers, are potential determinants of EAEC autoaggregation.

Naturally occurring rpoS polymorphisms modulate virulence gene expression in E. coli O104:H4

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Background
The alternative sigma factor RpoS regulates adaptation to varying ecological pressures in Escherichia coli (E. coli) and other Proteobacteria. We recently demonstrated that RpoS acts as a virulence repressor in E. coli O104:H4, causative agent of the 2011 outbreak of food-borne illness in Germany. In particular, we identified a laboratory-acquired single nucleotide polymorphism (SNP) in the rpoS start codon that reduced RpoS abundance, but significantly enhanced virulence gene expression.

Rationale
Here, we screened our collection of E. coli O104:H4 outbreak isolates for naturally acquired SNPs in rpoS and assessed their impact on virulence gene expression.

Main findings/outcomes
Analysis of whole genome sequence data revealed two naturally occurring SNPs in the C-terminal region of rpoS: 829C>T, introducing a premature stop codon and 836G>T, leading to a non-synonymous amino acid change. The mutated rpoS alleles were expressed heterologously in EHEC O104:H4 Δstx2 ΔrpoS and Western blot analysis demonstrated an association with reduced RpoS abundance, whereas rpoS829C>T also resulted in a truncated protein variant. Additionally, catalase assays revealed that SNPs led to complete loss of RpoS activity. Furthermore, they were linked to increased expression of aggregative adherence fimbriae type I, correlating with enhanced aggregation in liquid cultures and biofilms. Lastly, the expression of additional virulence factors such as SepA, a serine protease autotransporter, and the dispersin Aap were found increased by both SNPs.

Conclusions
Our findings highlight how naturally acquired rpoS mutations modulate virulence and ongoing transcriptomic analyses aim to further elucidate their impact on global gene expression in E. coli O104:H4.

Lytic phages MM-1 and MM-2 are active against Salmonella enterica and enterohemorrhagic Escherichia coli without phage-mediated Shiga toxin 2 induction

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Background:
Antibiotic treatment of EHEC infections is controversially discussed. One reason is the possible induction of phage-encoded Shiga toxins (Stx) by particular antibiotics. Lytic bacteriophages represent a promising alternative for biocontrol of EHEC.

Aim:
This study primarily aimed to isolate and characterize virulent bacteriophages active against EHEC strains. Furthermore, safety aspects such as a potential phage-mediated induction of stx2a expression should be investigated by qRT-PCR.

Main findings:
We isolated two tailed bacteriophages, vB_EcoS_MM-1 (MM-1) and vB_EcoS_MM-2 (MM-2), from a local sewage treatment plant using E. coli O157:H7 strain EDL933. Genome sequencing revealed that both phages contain linear dsDNA genomes of approximately 118,000 bp. Virulence and antimicrobial resistance genes have not been detected. The host range of MM-1 and MM-2 is not restricted to serotype O157:H7/H− but includes also other EHEC and Salmonella enterica serotypes. Both phages remain active across broad temperature (4–60 °C) and pH ranges (3–12) and exhibited a short latent period (15–20 min) with a burst size of appr. 90 PFU mL-1. They effectively inhibited growth of EDL933 at multiplicities of infection (MOI) of 0.1, 1, and 10. qRT-PCR analysis revealed no upregulation of stx2a expression after infection with either phage at any MOI.

Conclusion:
Phages MM-1 and MM-2 are promising biocontrol agents against EHEC, STEC, and Salmonella enterica strains, suitable for food safety applications and potential phage therapy. Ongoing work focuses on elucidating the molecular interaction between these phages and their bacterial hosts to identify factors that may limit or extend phage efficacy.

Use of chromogenic culture media in screening for STEC O26 in the management of nursery outbreak with cases of HUS.

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[1] UKHSA East of England Health Protection Team

In recent years there have been several reports of nursery outbreaks involving Shiga-toxin producing E. coli (STEC) O26 across the UK. Management of such outbreaks may involve closure of all or part of the nursery and screening of children prior to their return.

Screening is often undertaken using polymerase chain reaction (PCR) testing methods to detect the presence of stx genes. However, enteric PCR assays usually test for multiple pathogens at one time, and this can result in incidental findings of non-target organisms for screening and pose challenges for interpretation and risk assessment for Health Protection Teams.

In August 2023 the UKHSA East of England Health Protection Team managed a high-risk outbreak of STEC O26 in a nursery, with two cases of HUS. On advice from laboratory colleagues, the incident management team agreed to deviate from the usual practice of using PCR for screening and instead used chromogenic agar and latex agglutination to screen for further cases of O26.

Screening detected one further case of O26, confirmed via whole-genome sequencing to be linked to the other outbreak cases. Two cases of non-STEC were also detected. This method of screening was effective in returning results in a reasonable timeframe, without reporting a high number of alternative incidental findings.

Implementation of a novel One Health approach to support the investigation of the largest Shiga-toxin producing E. coli (STEC) O157 outbreak in the UK since the introduction of Whole Genome Sequencing

Dr Neil Cunningham[1]

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Background
Shiga-toxin producing E. coli (STEC) O157 is a foodborne pathogen associated with gastrointestinal illness and severe disease (34% hospitalisation rate, 5-15% develop haemolytic uraemic syndrome (HUS)). International studies have identified heavy rainfall as associated with STEC exceedance. In the UK, some strains of STEC are associated with gut carriage in sheep. In 2022, the largest UK outbreak of STEC O157 was identified since 2015. A national-level investigation was undertaken to investigate the cause, implement controls, and develop future intervention strategies.

Methods
WGS was used to identify outbreak cases of STEC O157. Food exposure information and food chain investigations were used to identify the likely source. Evidence generation included gathering information on crop rotation combined with analysis of meteorological and land use data.

Results
259 (STEC) O157 t5.5294 cases were confirmed across the UK. Descriptive epidemiological analyses supported salad produce as the vehicle. Contextual analysis of WGS data, international communications and case onset dates relative to supply chains supported a UK grown, nationally distributed food item with a short shelf life with salad leaf production by a single grower the likely cause. Weather [isolated and unusual heavy precipitation] and land use data [indicating land used to grow salad for human consumption] corroborated the location of the grower in an otherwise drought-like situation.

Conclusion
Due to the nature and potential severity of STEC infection, early detection and investigation is important. The use of One Health [including environment, land use and weather] data is a potential emerging tool for evidence generation and future mitigation strategies to prevent outbreaks.

STEC in Scottish sheep on farm and at slaughter

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[1] SRUC, [2] SERL, [3] BIOSS

Human STEC infections are linked to contact with ruminant faeces, or food and water contaminated with ruminant faeces. Cattle are well documented as sources of human infection, and sheep have been postulated and identified as source animals since the mid-1990s in Great Britain and continental Europe. However, very little is known about STEC carriage in Scottish sheep.

During 2022/2023, 875 sheep faeces were collected from farms and at abattoir in Scotland and STEC isolated from them were characterised by whole genome sequencing. The sheep sequences were compared with those in the SERL genomic database to place them in the context of Scottish human infections.
STEC O157 occurred at low frequency but clustered closely with human isolates; in some cases, close enough to indicate direct transmission or transmission from a common source.

Non-O157 STEC occurred more frequently, many clustered with human isolates and had characteristics associated with lower pathogenicity potential for humans.

The role of Scottish sheep as a reservoir for STEC infection may be complex, multifactorial, and dependent on serotype.

Absence of Shiga toxin-producing Escherichia coli in extended-spectrum-producing Escherichia coli genome sets derived from across Africa

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Background:
Shiga toxin-producing E. coli (STEC, including EHEC) cause major outbreaks globally, but their prevalence in Africa is poorly documented, with only a few low-resolution reports. We hypothesized that Tricycle and similar ESBL-focused surveys across humans, food chains, and the environment would detect endemic STEC if present. We screened genomes from WHO Tricycle projects in Nigeria and other African sites for STEC markers and compared them with global datasets.

Methods:
We analyzed ESBL-E. coli from the 2024 Tricycle surveillance in Nigeria (n=52) and 200 additional genomes from other African Tricycle projects. All genomes underwent quality-controlled (FastQC), screened for stx1, stx2, eae, and ehxA (MLST and VirulenceFinder), and sequence typed/serotypes with ectyper. We similarly reanalyzed WGS datasets from Madagascar and Ghana, and included comparable strain sets from Asia, the UK, and Europe for context.

Results:
We analyzed 252 ESBL-producing E. coli genomes from three African countries. All carried ESBL genes, mainly blaCTX-M. None contained stx1 or stx2, indicating no STEC/EHEC. Two isolates (1%) were typical/atypical EPEC with tir and other non-STEC diarrhoeagenic markers. Our findings align with existing evidence that STEC/EHEC is uncommon in Nigeria, Ghana, and Madagascar.

Conclusions:
Although limited to ESBL-positive genomes, our analysis of One Health ESBL-E. coli surveys in Africa detected no STEC/EHEC, strongly suggesting that STEC is not endemic in these settings. The risk of sporadic STEC infection in Nigeria and other African countries may be considerably lower than in many regions, though continued screening remains important as imported cases or point-source outbreaks can still occur.

How would you like your burger? Results from a survey and sequencing in the service of Scottish food safety

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Consumer behaviour is often driven by popular media and social trends, rather than meeting standards anticipated by existing food safety guidance and controls.

A 2019 microbiological survey of fresh beef mince on retail sale in Scotland aimed to: (1) determine the baseline prevalence of three foodborne pathogens, including Shiga toxin-producing E. coli (STEC); and (2) apply whole genome sequencing (WGS) to compare these with Scottish isolates from human clinical cases and cattle, to help inform risk assessment, management, and communication strategies.

Mince samples, purchased using a two-stage sampling strategy accounting for population density and market share of retail outlets, were tested for Campylobacter and Salmonella by culture, and STEC by PCR. PCR-positive samples were cultured to isolate STEC. WGS was performed on all isolates using the Illumina Miseq platform, and data were analysed using Scottish Microbiology Reference Laboratories' bioinformatic workflows.

Of 1009 mince samples, 226 tested positive for STEC by PCR with 35 organisms isolated and typed. WGS revealed serotype, antimicrobial resistance profiles and phylogenetic relationships. STEC isolates were classified by their potential to cause clinical disease in humans. Our results also provided microbiological evidence of a link between seven geographically dispersed human cases infected with the same STEC strain.

Our findings highlight the difficulties that arise when comparing prevalence estimates from different studies, due to a lack of standardisation in laboratory methodologies, and the relevance of sequencing to surveillance and control strategies. They demonstrate that regulatory controls contribute to food safety, while confirming the importance of consumer education.

Detection of Shiga toxin-producing Escherichia coli in Alberta, Canada

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Background:
Shiga toxin-producing E. coli (STEC) is a common foodborne pathogen and can cause severe clinical disease especially in young children. In Alberta, we have combined culture independent detection testing with enhanced culture methods to recover STEC isolates for serotyping, cluster analysis and surveillance.

Methods:
Stools submitted to Alberta Precision Laboratories-Alberta Public Health Laboratory (ProvLab) are extracted by eMAG®, followed by real time PCR to detect and differentiate between stx1 and stx2. All PCR positive samples are subjected to culture on CHROMagarTM STEC and MacConkey agar for isolation. Sweeps of each culture are assayed by PCR, and if positive, up to 15 individual colonies are picked for STEC confirmation by PCR and one positive colony is used for whole genome sequencing (WGS) using the ECTyper for serotyping. All sequences are also uploaded to the PulseNet Canada for cluster analysis/outbreak detection.

Results:
Total number of STEC from 2022 to 2025 June was 1476 with 365 (25%) E. coli O157 and 1111 (75%) non-O157. For non-O157 STEC belonging to the top 6 reported in this study period is 644 with O26 (n=228; 20.52%) as the predominant serotype followed by O103 (n=165; 14.85%) and O111 (n=131; 11.79%). Only one O45 serotype was detected in 2024.

Conclusion:
PCR and enhanced culture using 2 different agar media allows for effective detection and recovery of non-O157 STEC isolates. Serotyping using WGS and uploading data to PulseNet Canada supports the rapid detection of clusters/outbreaks throughout Canada.

Characterization of STEC strains isolated in flour and flour-based products, in France, between 2009 and 2024.

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Pathogenic Shiga toxin-producing Escherichia coli (STEC) are foodborne zoonotic bacteria associated with sporadic cases but also large-scale outbreaks that represent a major public health issue.

Historically, flours have been considered microbiologically safe products because they have low water activity and are generally intended to be cooked before consumption. However, cases of contamination have been reported, particularly in studies published in Germany, USA and Canada, confirming the possibility of transmission of the pathogen through this food chain.

French Food Busines operators in flour sector begun to implement programs for monitoring STEC in their products. The aim of this study was to provide a detailed description of the thirty-three STEC strains isolated from flour or flour-based products in France between 2009 and 2024.

We used Whole genome sequencing (WGS) to characterise strain serotypes, virulome and antibiotic resistance profiles. CgMLST comparisons were carried out for identical serotypes.

Thirteen different serotypes were isolated in flour and flour-based products, including nineteen strains belonging to serotypes O26:H11, O103:H2 and O157:H7. Only one strain showed antibiotic resistance genes. Certain strains possessed eae and stx genes, suggesting a high pathogenic potential. Our work showed that some strains isolated from products manufactured in distant regions of France and at different times, were closely related according to cgMLST. These data suggest persistence and potential circulation of strains.

It started with a pumpkin patch

Helen Corrigan[1], Rhona Kirkham

[1] Nhs Grampian

Background:
The NHS Grampian Health Protection Team (HPT) observed increasing cases of zoonotic infections associated with farm based visitor attractions. In 2021, a cluster of STEC cases was linked to a pumpkin patch, then followed 2 further genomic identical cases in 2022 with common exposure. The HPT initiated a joint visit with Environmental Health colleagues. This identified transmission points, mitigations were put in place and no further cases linked to this setting were reported. This approach was applied to other settings and pathogens with positive outcomes for both settings and visitors.

Rationale for the work:
To follow health protection principles of outbreak management and prevention of disease.

Main findings or outcomes:
We identified a lack of awareness around the risk of zoonotic infection from owners and visitors with reoccurring themes of lack of signage for hand washing, inadequate hand washing, reliance of hand gels, and lack of clear zoning between food, play and animal areas.

Outcome of working with providers and partners to better prepare settings for members of public visiting sites. Development of good practice guidance for providers to assist in above. The HPT along with partners and providers hosted a webinar to share learning and raise awareness of the good practice guidance document. Settings linked to previous cases have not had cases linked to their settings since having the joint input from HPT and EHO.

Conclusions:
Collaboration working, education and consistent messaging are pivotal in order to prevent future cases of zoonotic infections in humans.

Severe Childhood CKD and Kidney Transplantation Following STEC-HUS in Southern Alberta: A 25 Years Single-Centre Experience

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Background
Southern Alberta has one of the highest incidences of Shiga toxin–producing E. coli (STEC) infections in North America. While global registries identify STEC-induced hemolytic uremic syndrome (HUS) as an uncommon cause of pediatric end-stage kidney disease, we hypothesized this differs in our region.

Rationale
The Pediatric Nephrology Clinic at the Alberta Children's Hospital (ACH), the sole pediatric nephrology referral center in southern Alberta, has maintained records of all children with severe CKD and kidney transplantation over the last 25 years. Two regional studies reported high annual STEC-HUS incidence rates (2.6–3.3 per 100,000 children/year) with high acute dialysis requirements (45–52%). Our aim was to determine the frequency of severe childhood CKD and kidney transplantation attributable to STEC-HUS in southern Alberta and compare it with international registry data.

Main Findings
From 2000–2025, 184 children with severe CKD (stages 4–5) were followed, and 106 underwent kidney transplantation. STEC-HUS accounted for 9 severe CKD cases (4.9%) and 5 transplants (4.7%), exceeding the 1–4% reported globally. Based on historical incidence, we estimated approximately 215 STEC-HUS cases during this period, with roughly 97 requiring acute dialysis. Applying published long-term kidney sequelae rates (~25%) and excluding childhood cases, we estimate that 40 additional individuals from this cohort are at risk of developing CKD later in life, with some progressing to ESRD.

Conclusions
Southern Alberta shows a higher contribution of STEC-HUS to severe childhood CKD and pediatric kidney transplantation than reported internationally, highlighting the importance of prevention, early recognition, and long-term follow-up of STEC-HUS survivors.

Detections of STEC in raw pet food at retail sale in the UK

Dr Frieda Jorgensen[1]

[1] UKHSA, [2] UKHSA

Raw pet food (RPF) has in previous studies shown to harbour STEC. Raw dog and cat food samples (n = 380), from 50 brands were sampled in the UK, March 2023 to February 2024. STEC were detected in 11.8% of samples and PCR-based testing of enriched samples detected stx genes in 48.4% of samples with 32 different serotypes were identified including STEC O26:H11, O128ab:H2, O76:H19, O166:H28 and O113:H4. Some isolates carried stx1a (1), stx1c (6) and stx1d (1), stx2a (1), stx2b (6), stx2d (5), stx2e (2) and stx2g (9) and combinations: stx2a and stx2e (2), stx2c and stx2d (1), stx2b, stx2c and stx2d (1) and 13 isolates had combinations of stx1 and stx2. Considerable diversity in the STs was detected (41 STs). The isolates were not closely related. The majority (88.9%) were predicted to be susceptible to all antimicrobials but one ST10 had an AMR profile of: blaTEM-1, strA, strB, aph(6)-Id, gyrA(S83L), mph-B, dfrA-1, sul-1, sul-2, tetA and floR and one ST329 isolate had aadA-1b, ant(2'')-Ia, mph-B, gyrA(S83L), dfrA-1, sul-1,sul-2, tetM and floR profile. It is significant that the STEC isolated here were all of ST types capable of causing human illness and 11 isolates harboured stx2a, stx2d and/or stx1a known to be associated with more severe clinical outcome. Considering the low infectious dose and potential severity of disease, the occurrence of STEC in raw pet food samples poses a health risk for persons handling RPF and persons with close contact to pets fed RPF.

The WHO Alliance for Food Safety: Enhancing Surveillance and Laboratory Capacity for Safer Food Worldwide

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The World Health Organization (WHO) has established the WHO Alliance for Food Safety to work collaboratively to implement the WHO Global Strategy for Food Safety (2022–2030) with a focus on enhancing national foodborne disease (FBD) surveillance and food contamination monitoring worldwide. The Alliance aims to create an enabling environment for multisectoral collaboration, advocate for and promote legal frameworks supporting FBD surveillance, map and build laboratory capacities, and facilitate the generation and sharing of high-quality data and best practices.

One of the Working Groups of the Alliance (focused on laboratory capacity building) is instrumental in achieving these goals by aiming to strengthen national public health, animal health, and food analysis laboratories. These efforts contribute to more effective food safety interventions, improving the detection and control of foodborne pathogens such as diarrheic E. coli. Here Shigatoxin-Producing E. coli (STEC) are the most prominent one.

Strengthening STEC surveillance as part of a robust FBD surveillance system is, therefore, a cornerstone of global food safety efforts. WHO supports countries in enhancing their laboratory capacities for detection and isolation, fostering harmonized methodologies, and improving data sharing at the regional and global level.

By reinforcing laboratory networks, improving surveillance systems, and promoting best practices among Alliance members and beyond, the WHO initiative aims to reduce illnesses due to STEC and contribute to safer food systems worldwide. This collaborative approach contributes to national authorities being better prepared to prevent, detect, and respond to foodborne outbreaks, ultimately protecting public health and reducing the global impact of foodborne pathogens.

Surveillance of Shiga-toxin producing E. coli (STEC) infections in children with bloody diarrhoea: experience from southern Italy

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Background
STEC infections represent a significant public health threat and can cause bloody diarrhoea (BD), potentially leading to haemolytic-uraemic syndrome (HUS), especially in children.

Rationale
In 2018, following an abrupt increase in HUS cases, surveillance of STEC was implemented in Southern Italy and later expanded with genomic surveillance. This study describes the results of STEC surveillance in children aged ≤16years with BD (June 2018-November 2025). STEC infections were identified through rapid diagnosis and WGS was conducted on isolates. Sequences were uploaded on the national platform ISS-IRIDA-ARIES.

Main findings
A total of 320 STEC infections were detected out of 4,259 BD cases. Median age of STEC cases was 2 years (IQR:1-6). The average reporting rate was 0.8 cases/10,000 resident children. Serogroups O26 and O157 were the most frequently recorded (24.7% and 23.4%, respectively). The virulence gene eaeA was detected in 77.8% while stx2 and stx1 were detected in 60.3% and 56.6% of STEC strains, respectively. A progression to HUS was recorded in 10.0% of cases, the majority of which was sustained by a stx2-producing strain. No deaths were reported. Genomic analysis on the ISS-IRIDA-ARIES platform revealed that 25 STEC strains were linked to 12 clusters, one of which was correlated with a multi-country outbreak.

Conclusions
Surveillance showed persistent STEC circulation from 2018 to 2025 in the region, particularly of O26 and O157 serogroups. Moreover, surveillance allowed prompt patient care and improved management of HUS cases. A risk-based, multi-disciplinary approach is crucial for surveillance of STEC infections and improvement of HUS outcomes.

Prevalence and Genetic Diversity of Shiga Toxin-Producing E. coli (STEC) in Finnish Beef Production

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Background
Beef is a significant source of Shiga toxin-producing Escherichia coli (STEC) infections in humans, primarily due to carcass contamination during slaughter. In Finland, monitoring has focused on STEC O157:H7 since 2004, but the program was recently expanded to include all STEC types. This study examines data from a 2020 pilot project testing new sampling strategies and compares results with national control program data spanning 2021–2024.

Rationale for the Work
The aim was to assess STEC prevalence, genetic diversity, and virulence in Finnish beef production, evaluating contamination routes and consumer health risks. Whole-genome sequencing (WGS) was applied to characterize isolates and determine their pathogenic potential.

Main Findings or Outcomes
In the pilot study, culture-confirmed STEC was detected in 11% of carcass and 14% of bovine fecal samples, but not in meat. During 2021-2024, the prevalence in carcasses varied between 10.5% and 14.6%. WGS of 283 isolates revealed 49 MLST types and diverse serotypes, with only 8.5% belonging to top five serogroups. Most isolates (58%) carried the stx2 gene, and 14% exhibited hybrid STEC-ETEC characteristics. Nearly 45% of isolates harbored virulence genes associated with severe human disease; in the pilot study, such strains originated from fecal samples.

Conclusions
STEC occurrence is common in cattle and carcasses. The pilot study indicated rare meat contamination, suggesting effective hygiene measures during slaughter.
In conclusion, the variety and virulence of STEC strains underscore the need for continuous surveillance and regular fecal sampling to comprehend STEC population genetics and ensure public health safety in Finland.

The outbreak that never was: investigating a surge in STEC notifications in NHS Fife in September 2025

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Background:
In September of 2025 we detected a rise in STEC notifications in NHS Fife.

Methods:
Confirmed and probable STEC Cases notified over an 8 week period were identified, their demographic, surveillance and laboratory information combined and the findings discussed at a multiagency Problem Assessment Group (PAG).

Findings:
Sixteen cases were identified. In 2 cases (12.5%) there had been co-traveller exposure and in 2 cases (12.5%) household transmission was assumed. However just over half of the cohort (n=9, 56%) were categorised as community transmission of unknown source.

Review of surveillance data found that there were 3 local settings which were named on more than form. Of note one of the food shops found to be a local chain at which 2 cases purchased pork sausages 6 weeks a part and shared the serogroup E Coli O91:H14. However subsequent Whole Genome Sequencing (WGS) cluster analysis by the reference laboratory confirmed these to be different strains.

Of the 12 cases for whom more detailed laboratory findings were available at time of investigation no samples were found to be related to each other.

Conclusions:
The findings were interpreted in the context of local knowledge at a multiagency PAG. Out with the 4 cases deemed to be travel associated and/or household transmission, we concluded there was no evidence of an outbreak which connected these cases in space, time or by laboratory findings. A high prevalence (56%=9) of pet ownership and more sensitive laboratory testing locally in NHS Fife were felt to be contributory factors.

Comparative genomics of two stx2f STEC O80:H2 isolated from diarrheic calves in Belgium and belonging to different lineages

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BACKGROUND.
Attaching-Effacing (AE) and Shiga toxin-producing Escherichia (E.) coli (AE-STEC) O80:H2 have been emerging in humans in Western Europe since 2010. In parallel, AE-STEC and enteropathogenic E. coli (EPEC) O80:H2 have been more frequently isolated from young calves in Belgium since 2009. All 127 human and calf AE-STEC and EPEC isolated between 2008 and 2024 in Belgium are intermixed in the L1 lineage of a Single Nucleotide Polymorphism-based phylogenetic tree with 124 isolates present in two major sub-lineages (SL1.1 with 60 isolates and SL1.2 with 64 isolates), while two calf AE-STEC isolated in 1987 form the L2 lineage.

RATIONALE.
The purpose of this study was to compare the genomics of two stx2f calf AE-STEC belonging to SL1.1 (SES0057) and SL1.2 (SES0108) after long-read sequencing.

RESULTS.
Both isolates harboured a Locus of Enterocyte Effacement carrying the eaeξ gene, two Stx2f phages and a pS88 plasmid. The two Stx2f phages of SES0108 were >99% identical to Stx2f phages described in the literature, while those of SES0057 were different. The pS88 plasmid of SES0108 carried the etsC and iucC genes, while the pS88 plasmid of SES0057 did not, but carried the cma gene. Conversely, the iha gene located in the chromosome-integrated “Sakai-prophage like element 1” was detected only in strain SES0057.

CONCLUSIONS.
Detailed analysis of the genomic differences is important for defining the structure of the AE-STEC and EPEC O80:H2 population and understanding the evolution of the different lineages, not only in Belgium, but also in other European and non-European countries.

Verotoxigenic Escherichia coli shedding in Ireland: Duration and associations

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Ireland has one of the highest incidence rates of VTEC in the European Union. Every year, many people in high-risk groups are excluded from work, school and childcare pending VTEC clearance of two negative stool samples taken at 24 hours apart. This study aims to determine the duration of shedding in Irish VTEC cases and to identify risk factors associated with persistent shedding.

Data on 2889 culture positive VTEC cases received at NRL-Dublin between 2017 and 2019 was extracted from the Laboratory Information System. Cases that were culture positive and where microbiological clearance was achieved were included in the analysis.

340 cases with microbiological clearance were identified. 208 (61.2%) cases were female, 130 (38.2%) were male, and 2(0.6%) of unknown sex. Age ranged from 0-82 years, median 2.8 years. Children (0-14 years) comprised 83.2% of cases, adults (>14 years) 16.5% of cases, and in 0.3% age was unknown. VTEC shedding ranged from 3 to 479 days (median, 28 days; interquartile range, 25 days). Prolonged shedding was defined as a shedding period longer than the median. Children shed VTEC for longer than adults ($p<0.001$), however there was no significant correlation between shedding duration and sex, serogroup, major virulence genes or stx subtype.

Age was the only risk factor associated with prolonged shedding of VTEC. A median shedding duration of 28 days leads to reduced productivity and increased economic burden among caregivers. However, with shedding not being associated with VTEC strain, it is not possible to predict shedding duration for individual cases.

Rapid typing of STEC O157 by IS629-printing during a nursing home outbreak in Belgium, August 2025

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In August 2025, Belgium experienced an unprecedented outbreak of Shiga toxin-producing Escherichia coli (STEC) O157 in multiple nursing homes (NH), raising significant public health concerns. A total of eleven NH were affected across Flanders (9), Brussels (1) and Wallonia (1), with 66 suspected cases and 10 reported deaths. Epidemiological investigation and food consumption analysis identified raw minced beef as the most likely source of infection.

In response to this suspicious epidemiological cluster, IS629-printing was applied as primary rapid typing method for STEC O157 at the Belgian National Reference Centre (NRC). This approach allows preliminary cluster confirmation as stool specimens – at least one per affected NH – are progressively referred to the NRC. The rapid results facilitate timely communication with public health authorities, complementing the (bi)weekly batch processing of whole-genome sequencing (WGS) data.

All NH isolates were characterized as STEC O157:H7, stx1a+, stx2a+ and eae+, and shared identical IS629-profiles, strongly suggesting a single cluster. One isolate from a NH, which had no apparent link to the common source, was excluded from the outbreak based on a distinct IS629-profile. One community isolate from a case who consumed raw beef, shared the same IS629-profile as the outbreak isolates. Core genome multilocus sequence typing analysis confirmed that all genomes with identical IS629-profile belonged to the same HC5_357459 cluster.

The obtained results stress the utility of IS629-printing as primary rapid typing method for STEC O157 at the Belgian NRC, enabling preliminary cluster confirmation and timely communication with public health authorities pending definitive WGS confirmation.

Mind the gap! Typing E. coli O157:H7 in the pre- and post-genomic revolution eras

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Background
Over the last decade, whole genome sequencing (WGS) has replaced phage typing (PT) as the typing method of choice to detect outbreaks and monitor trends of STEC O157:H7. We explored correlation of historical PT data with WGS outputs, to enable us to continue to describe trends in STEC O157:H7 notifications in England over a 40-year period from 1985 to 2024.

Rationale
Since the 1980s, in the UK we observed the emergence of three dominant phage types, PT2, PT21/28 and PT8, most likely driven by the acquisition of bacteriophage encoded stx2a, known to be associated with more severe clinical outcomes, and more efficient transmission of STEC O157:H7 within the animal reservoir. We aimed to determine whether PT and Shiga toxin (stx) profile could be used as a proxy marker of WGS-derived lineage and sub-lineage.

Main findings
Between 2016 to 2021, 3,229 isolates of STEC O157:H7 were linked to PT, stx subtype and WGS data.

We showed that PT2/stx2, PT21/28/stx2, PT8/stx2 and PT8/stx1+stx2, the four dominant types of STEC O157:H7 in the UK, correlated with lineage I/II (56.5%), and sub-lineages Ic (99.2%), IIb (82.1%) and IIc (99.3%), respectively.

Conclusions
Linking PT to sub-lineages enables us to monitor and determine the drivers of the overarching trends over a 40-year time frame spanning the pre- and post-genomic revolution era. Using these data, we can predict, investigate and prepare for emerging threats to public health, establish sub-lineage, specific animal reservoirs and transmission routes, and learn from previous outbreak investigations.

Detection of STEC strains producing different Shiga toxin subtypes by new immuno-nanoparticle culture media IDEA™ STEC

DAVID TOMAS[1], Lisa SCIANDRA[1], Benoît MALLEN[1], Thomas JUNILLON[1]

[1] Biomérieux

STEC isolation is a challenging step, with low percentages of confirmed results. New culture media has been developed using nanoparticles covered by specific antibodies (IDEA™ STEC agar) targeting stx and eae positive strains by producing clear surrounding halo around isolated colonies, indicating shiga toxin production. In this study, we evaluated the performance of IDEA™ STEC agar to detect STEC strains producing different shiga toxin subtypes. A total of 46 STEC strains from clinical samples, as well as positive and negative controls, were tested by Statens Serum Institute (Denmark) on IDEA™ STEC agar.

Results from TOP 7 STEC serogroups (O157; O26; O111; O145; O103; O45; O121) showed positive detection for 21 out of 24 strains tested. Three out of four strains from O80 and the sole included O104 strain were also positive. Positive results were observed for strains producing different stx subtypes alone or in combination (stx1a; stx1b; stx1c; stx2a; stx2b; stx2c; stx2d), except for the four strains tested producing the stx2f subtype. From 24 strains containing stx and eae genes, (typical EHEC) only four were negative: O121 (stx1a); O45 and O63 (stx2f) and O80 (stx2a). Tests performed with 1/10 dilution and presence of background flora showed equivalent results, not impacting the recovery of the target strains.

The new media allows an easy identification of colonies covering a broad range of stx subtypes including typical strains EHEC (stx and eae positive) and different serogroups. Further improvements will be required to detect shiga toxin production from all STEC strains.

Shiga toxin-encoding Escherichia coli from South American Camelids in Germany – prevalence, stx gene subtype distribution and strain characterization

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South American camelids (SAC) are popular in Europe, frequently kept with other livestock species and in close contact with humans. They represent a potential reservoir for transmission of epizootic and zoonotic bacteria to livestock and humans. However, knowledge on bacterial pathogens in SAC is too sparse for drafting appropriate monitoring and preventive medicine programs. To investigate the presence of Shiga toxin-encoding Escherichia coli (STEC) in SAC, 20 animals each were sampled four times in ten or nine herds. The prevalence of stx-positive samples was 32.4%. This resolved into 6.4%, 18.4% and 7.5% prevalence for stx1-, stx2- and stx1/stx2-positive samples, respectively. There was no difference in prevalence between alpacas (43.1%) and llamas (41.1%). The herd prevalence diverged widely from 0% to 95% stx-positive animals in the herds. The stx-subtypes identified were predominantely stx1c (78%) and stx2b (77%), while a few animals were PCR-positive for stx1a, stx2c, stx2d, stx2e, stx2f and stx2g. Multiple stx2 subtype signals were detected in several samples (13%). In 27 stx2-positive samples, no subtype could be assigned. So far, five isolates were obtained and sequenced. Three share the genoserotype O87:H16, one the genoserotype O151:O118:H16 and the remaining isolate has the genoserotype O76:H19. The herd prevalences determined here are similar to prevalence ranges published for cattle, small ruminant and camelid herds. Colonization with STEC differed widely between individual farms, but showed no seasonality. More isolates will be required to assess the public health risk of STEC from SAC. Those identified so far would classify as having low pathogenic potential.

Health inequalities: The impact of public health exclusions on cases of STEC infection and their contacts.

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Background

Scotland reports a higher burden of Shiga toxin-producing Escherichia coli (STEC) than many comparable countries. National guidance recommends exclusion until microbiological clearance for cases and contacts in defined high-risk groups. Following the introduction of local gastrointestinal PCR testing in NHS Tayside, a marked increase in STEC detections and associated exclusions highlighted apparent inequalities. This study aimed to assess whether exclusions disproportionately affected specific demographic groups.

Methods

All confirmed O157 and non-O157 STEC cases notified to the NHS Tayside Health Protection Team between 1 April 2022 and 31 March 2024 were reviewed. Demographic characteristics, exclusion rationale, work/care setting involved, and exclusion duration were extracted for cases and contacts, including adults excluded solely to provide childcare. Data were anonymised and analysed descriptively.

Findings

Across 138 confirmed cases, 87 individuals required exclusion from childcare or workplace settings. Children accounted for over half of exclusions with the longest durations (median 29 days). Adult exclusions predominantly affected women (90%) many of whom worked in lower paid roles. A substantial proportion of exclusions were associated with STEC strains that would not meet exclusion criteria under guidance used in other comparable countries such as England.

Conclusions

STEC related exclusions disproportionately affected young children and adult women. Increased detection of low risk strains amplifies the social and economic impact of exclusion policies, underscoring the need for proportionate, risk-based approaches that maintain effective infection prevention while minimising unnecessary disruption. Further analysis incorporating an additional two years of data is planned.

Genome variation within a CC32 O145:H28 stx2a Shiga-toxic Escherichia coli strain responsible for recurrent annual outbreaks in the UK.

Mr Jake David Turnbull[1], Dr David Greig[1], Dr Claire Jenkins[1]

[1] UK Health Security Agency

Background:
In May 2024, a large outbreak of over 300 cases of foodborne gastrointestinal illness occurred in the UK caused by STEC O145:H28. Phylogenetic analysis identified smaller, recurrent clusters of the same strain in 2022, 2023 and 2025. We interrogated the accessory genomes of strains (n=17) from each annual cluster to look for microevolutionary events within the outbreak strain.

Rationale:
Using long read data sequenced on the Oxford Nanopore platform, we conducted a longitudinal analysis of genome variation in an annually detected core genome 5-SNP cluster (designated t5.206), not mediated by the accumulation of vertically inherited SNPs, to better understand genome plasticity in STEC within an outbreak context.

Main findings or Outcomes:
A highly conserved 87kb IncFIB/IncI plasmid was identified within all isolates. Chromosomal inversions of 240kb to 1.4mb in size were detected flanked by prophage. The 240kb inversion was observed each year in the same location in the genome. Of the two larger inversions, one occurred in an isolate from 2023, and the other in an isolate from 2024. Prophage content was largely consistent between isolates over time, with three instances of the loss of a single prophage region.

Conclusions:
Over the 4-year time frame the outbreak isolates examined here exhibited conserved plasmid and prophage content, although chromosomal inversions were detected both intra and inter-annually. Prophage reshuffling across the accessory genome may play a key role in niche adaptation, by driving large chromosomal rearrangements that facilitate survival and persistence of re-occurring outbreak strains over time.

Shiga toxin 2a subunit production in enterohemorrhagic Escherichia coli deviates from the canonical 1:5 ratio

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Introduction:
The observation that A-subunits of Shiga toxin 2a intoxicate eukaryotic cells in the absence of the corresponding B-subunits raised the question whether the toxin subunits are produced by the bacteria in a strict 1:5 ratio, or whether the A-subunit is produced in excess and is released into the bacterial environment.

Rational of the work:
The aim of this study was therefore to investigate a possible molecular background for the occurrence of free Stx2a A-subunits in EHEC O157:H7/H-, O26:H11, O103:H-, O104:H4 and O113:H21 strains.

Main findings:
Transcriptional analysis of the Stx2a subunit gene expression showed that the A-subunit gene was expressed on average 1.90 times stronger than the B-subunit gene, indicating the presence of free A-subunits. Using Western blot analysis, free A-subunits were indeed detectable in the culture supernatants of all six strains. To compare the transcription ratios with the amount of subunit proteins present in the culture supernatants, a quantitative Stx2a subunit-specific ELISA was used. With this assay, two groups of strains with StxA2a:StxB2a subunit ratios of 1.10 and 4.63 were found.

Conclusions:
The results of this study demonstrate that Stx2a A- and B-subunits are not produced in a 1:5 ratio and that free A-subunits can occur without corresponding B-subunits. As a consequence of this phenomenon, the role of free A-subunits for EHEC-mediated pathogenesis should be investigated in future.

Evaluating the Presence of STEC O26 in Midwestern Agricultural Areas

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Background:
Cases of human disease caused by non-O157 STEC serogroups, including O26, have been on the rise globally. Sharp increases in O26 case counts have been observed in many European countries in recent years, and in some regions, O26 has surpassed O157 as the primary cause of HUS. These shifts coincided with the emergence of O26 sequence type (ST) 29, which primarily carries stx2 alone. While these trends have been characterized in many countries, we lack a detailed understanding in the United States.

Rationale:
We assessed the presence of STEC O26 in the Upper Midwest region of the U.S. (Minnesota, Wisconsin, Iowa, North Dakota, and South Dakota), a region with substantial agricultural activity. We utilized county-level case data captured by the CDC's PulseNet surveillance system from 2010-2023, as well as various agricultural covariates, to assess the risk of O26 infection in the region. Additionally, we incorporated genomic data to investigate the regional presence of O26 ST 29.

Main Findings:
We identified 716 O26 cases in the region over the study period. While case counts trended up over time, there was no marked increase. Only 34 of these were identified as caused by ST 29 strains, suggesting limited local establishment. Preliminary analyses found statistically significant relationships between O26 cases and multiple covariates, including agricultural worker counts and ruminant counts.

Conclusion:
STEC O26 is a unique pathogen with associations that appear to deviate from other prominent serogroups. This emphasizes the importance of targeted investigations to characterize the risk posed to communities.

Shiga Toxin Subtype 2f: A Frequent STEC Subtype in Denmark

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[1] Statens Serum Institut

Since it was first described, shiga toxin subtype 2f (stx2f) has become a prominent subtype among Shiga toxin-producing Escherichia coli (STEC) isolates submitted to the Danish reference laboratory at Statens Serum Institut. Because stx2f is rarely associated with severe disease or haemolytic uremic syndrome (HUS), surveillance often prioritises subtypes with higher virulence potential. However, the high prevalence of stx2f among the Danish STEC patient isolates warrants a closer examination of its clinical importance.

We reviewed national STEC surveillance data from 2017 to 2024. Isolates referred from primary diagnostic laboratories underwent whole-genome sequencing, and virulence profiles and serotypes were determined in silico. Referral patterns shifted over these years, with an increasing focus on subtypes associated with HUS (stx2a, stx2d), introducing selection bias. Despite this, stx2f was the fourth most common subtype, accounting for 11% of isolates with assigned stx subtypes (293/2676), following stx1a (20%), stx2b (17%) and stx2a (13 %). Stx2f was not observed in combination with other stx subtypes, was most frequently associated with serotypes O63:H6 (48%), O145:H34 (13%), O125:H6 (12%), O132:H34 (5%) and O113:H6 (3%), and predominantly observed in eae-positive isolates (95%). When stratified by age, stx2f displayed a notable prevalence among one-year olds (<1yo: 29, 1yo: 97, >1yo: 167), as the dominant subtype for this strata.

Stx2f represents a frequent STEC subtype in Denmark, even within a surveillance system increasingly biased toward more virulent subtypes. Its overrepresentation among young children and the high prevalence of eae suggest that stx2f may play a more substantial role than previously recognized.

New Method for Detection and Isolation of STEC O80:H2 in Foods

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Background:
STEC O80:H2 strains belonging to ST301 have been identified as a significant public health problem, owing to their association with severe human infections. Such strains own the distinctive feature of exhibiting a mutation affecting the utilization of melibiose as well as various antibiotic resistance genes.

Rational of the work:
STEC detection in food matrices is often hindered by low contamination levels and the presence of competing background flora. In this context, the objective of our study was to establish a two-step workflow for O80 :H2 STEC detection and confirmation suitable for routine analysis of foods.

Main findings and conlusions:
Initialy, we designed a multiplex RT-PCR targeting simultaneously O80-specific gene and the characteristic mutation of melibiose. The RT-PCR was validated using reference strains and spiked food samples. It showed high sensitivity and specificity, enabling rapid and reliable detection of O80:H2 ST301 STEC strains in enriched food matrices.

Subsequently, selective agars media were developed to improve the confirmation by isolation of suspect samples. Those media contained specific antibiotics to inhibit background flora and melibiose as a differential carbohydrate for phenotypic differentiation of ST301 strains. Agars evaluation on enrichment broths of raw milk cheese, minced beef, flour and vegetables demonstrated good discriminatory capacity and consistent target strain recovery.

In general, the combined use of multiplex RT-PCR and selective melibiose agar is an excellent strategy for O80:H2 ST301 STEC detection and monitor these strains in foods.

Detection of Shiga toxin-producing colonies on IDEA STEC Agar: First insights by using genomically characterized dairy isolates.

PhD Joerg Hummerjohann, Mrs. Elvira Wagner

[1] Agroscope

One of the bottlenecks of the current STEC detection methods is the colony isolation. Recently, the IDEA STEC agar designed to isolate mainly stx+ and eae+ carrying strains based on Shiga toxin production was launched by bioMérieux.

The aim of this study was to gain first insights around performance of IDEA STEC agar by using STEC dairy isolates, which have be characterized by WGS.

25 clonally not related STEC isolates with the following key characteristics where plated on IDEA STEC agar from overnight cultures in TSB at 37°C: 14 different serotypes including O26:H11 and O174:H2 as the most frequent ones, 19 isolates were stx1+ and 12 stx2+ including 8 different stx-subtypes with stx1a and stx2d as the most frequent ones. Ten isolates were stx+ and eae+.

17 out of 25 STEC isolates were found to be positive for Shiga toxin-production (presence of halo) including 3 weak halo formers. 15/17 stx1a+ carriers and 4/5 stx2d+ carriers where Shiga toxin-producers (some of them carried other stx-subtypes in the same cell). Remarkly, according to the intended use, all stx+ and eae+ isolates were positive for IDEA STEC agar .

Our first insights trial indicates that IDEA STEC agar can facilitate the detection of STEC directly on plates, especially eae-positive strains. Further studies can help to confirm the observed trends, and reach deeper conclusions, e.g. using a higher number with more different genomically characterized STECs.

Effect of Mitomycin C on Shiga toxin-producing Escherichia coli (STEC)

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Background:
In Alberta, PCR along with Enzyme immune assay (EIA) are used to detect STEC. However, as PCR is more sensitive than EIA, discordant results are frequently observed. To address this, we tested whether pretreatment of clinical samples with Mitomycin C (MMC) to induce the production of Shiga toxins could increase the sensitivity of EIA.

Method:
To determine the optimal concentration of MMC (5 to 500 ng/mL) for Stx induction, 3 distinct clinical STEC strains were used: O5:H19 (stx1c), O26:H11 (stx2a), and O157 (stx1a/2a). PCR and EIA assay were performed after overnight broth enrichment with/without MMC. After determining the optimal concentration, MMC was used as an enhancer for toxin production on 53 STEC isolates. Finally, 15 clinical stools were tested after broths enrichment in the presence/absence of MMC.

Results:
At 500 ng/mL of MMC, STEC isolates did not exhibit growth inhibition. EIA detected 44/53 (83%) of the isolates. After MMC treatment, EIA detected an additional 4 isolates, bringing the total number isolates detected by EIA to 48/53 (90%). These were distinct serotypes/ toxin types. Of the 15 clinical stools detected by PCR, 10 were positive by EIA and MMC treatment had no effect.

Conclusion:
MMC enhanced toxin production in most STEC strains, especially those with Stx2. However, some strains responded poorly to MMC regardless of the stx type. More research is needed before MMC can be used as an enhancer for toxin production to improve STEC detection in diagnostic laboratories.

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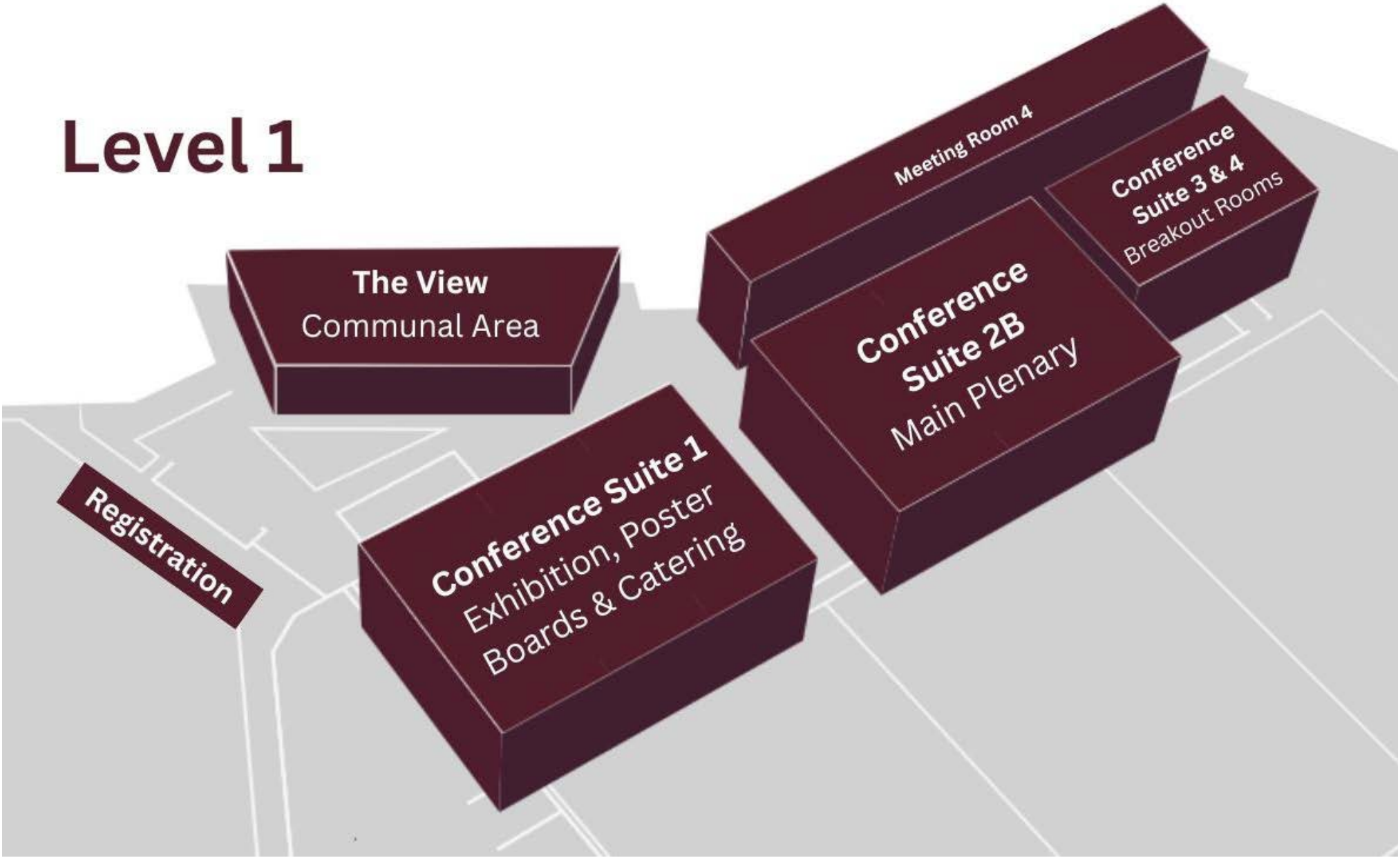
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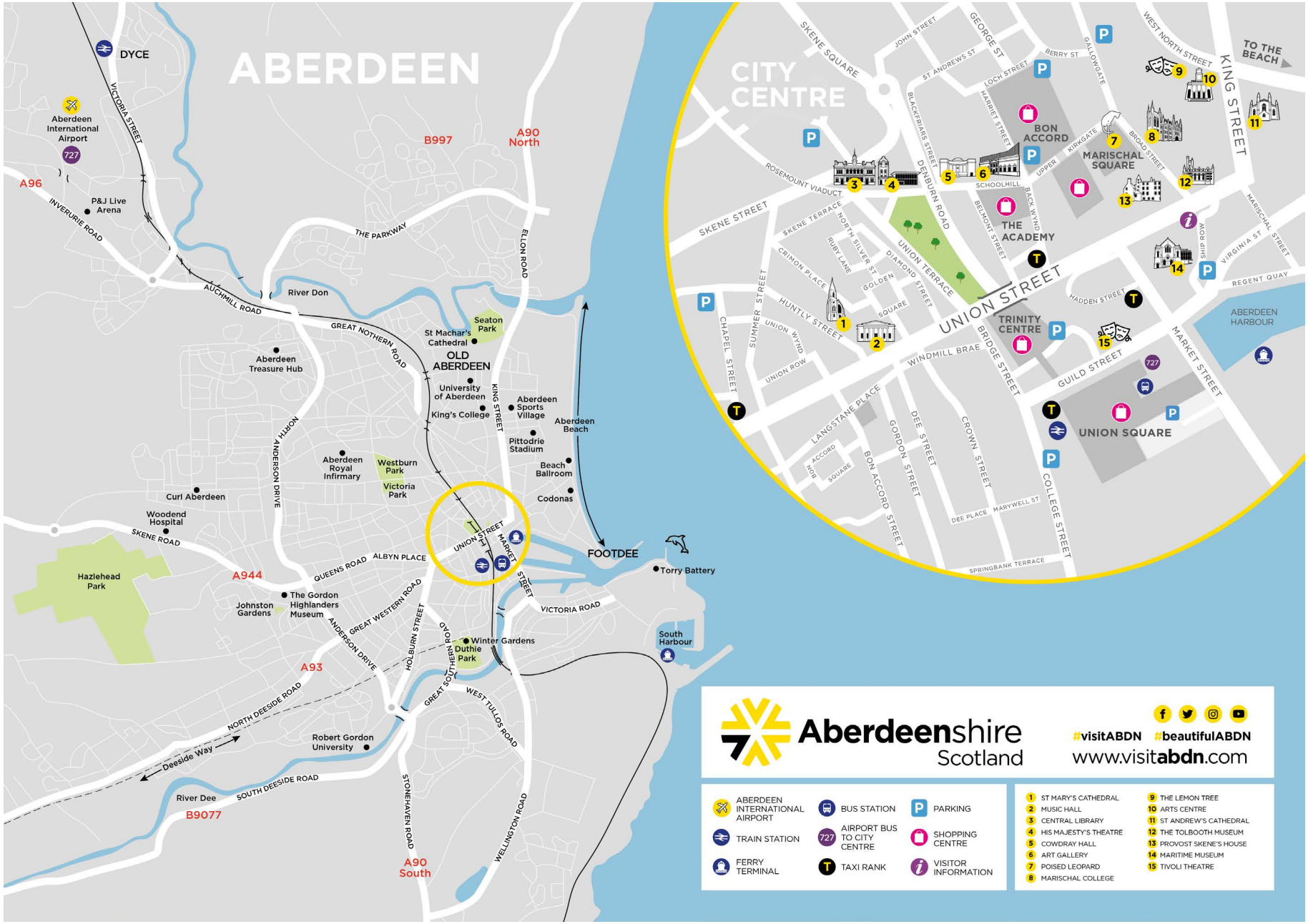
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