Summary of CoZEE 2014 meeting and workshop: "The role of high-throughput sequencing in surveillance, diagnostics and tracking zoonotic"

11-12 November 2014

The James Hutton Institute, Dundee, UK

Contents

Introduction

A meeting and workshop was held in 11-12 Nov 2014, Dundee to discuss the role of high throughput sequencing approaches in surveillance, tracking and diagnostics relevant to zoonotic pathogens. The topic for the meeting and discussion was reached by the CoZEE steering group: it was considered to be topical and of particular relevance to public health agencies (FSA-S, HPS and public health laboratories and similar agencies elsewhere) in their decision making going forward. The FSA had previously held a similar workshop in Jan 2012 "The Application of Molecular Epidemiology to Investigations of Foodborne Disease Outbreaks: Current Status and Future Plans" and were keen to explore developments in the field that were of particular relevance to Scotland. CoZEE was also keen to ensure that the topic was open to all groups that have a relevant interest in zoonotic pathogens.

[Note: The sequencing approaches are referred to as interchangeably as Next Generation Sequencing (NGS) or whole genome sequencing (WGS), although it is noted that novel technologies are superseding already established ones, and in many instances only a portion of the total amount of sequence data is used in practice.]

Summary and main conclusions

The meeting was designed such that invited presentations were given covering a range of topics relevant to the area, in order to appraise on current status and to inform the breakout sessions. The invited talks were supplemented by offered presentations and posters. The meeting was well attended with representatives from stakeholder groups including FSA, PHE, reference laboratories (SERL, SSSCDRL) and testing laboratories (Tayside Scientific Services, Edinburgh and Glasgow city council labs), MRPs (James Hutton Institute, Moredun Research Institute) and academic institutions from the UK and abroad (Ireland, USA, Germany).

The focus of the presentations and discussion was on bacterial pathogens with the presentations illustrating how sequencing approaches are developing and case studies showing how they can be used in diagnostic/surveillance settings as well as research. Aspects addressed included limitations with data acquisition, management and interpretation. Discussion groups addressed 4 key issues: (i) Tracking Origins and Destinations; (ii) Polymorphisms and consequences for transmission/virulence; (iii) Resources and provisions; and (iv) Bioinformatics Applications and Considerations. The main conclusions from the discussion groups are:

- There is a growing role for sequencing as a central approach in each of the aspects of diagnosis, surveillance and tracking of zoonoses during outbreaks.
- Significantly, specific applications need to be appropriate to the pathogen and to the requirements for appropriate epidemiological typing, e.g. the possibility of using *in silico* versions of established techniques such as MLVA 'vs' SNP detection and analysis, or defining appropriate gene-sets for discrimination.
- Limitations in current capacity in bioinformatics, data handling and analysis were raised as key considerations.
- Consideration also needs to be given to associated metadata, both in terms of its usefulness and any issues relating to privacy or otherwise.
- There is a fundamental requirement for collaboration and data sharing as well as data standards/standardisation, accessibility and ownership. e.g. minimum data necessary to for meaningful portability and utility between reference laboratory and research groups (and vice versa).
- A centralised sequencing resource(s) in Scotland focussing around outbreak control may overcome many of the issues, e.g. in sequence capability and bioinformatics expertise. It was noted that in Scotland, we already have well established networks between different institutions, and the size of the country is well suited to promote efficient community working.

Stakeholder presentation summaries

A brief summary of the presentations with some of the key points highlighted.

David Ussery (Center for Genomic Epidemiology, Technical University of Denmark. Currently at Oak Ridge National Laboratory, USA) showcased developments in sequencing technologies comparing short-read datasets to '3rd generation' sequencers that give long reads, which has profound implications for sequence assembly. He also raised the possibility of sequencing directly from samples without the need for culture. Finally, David showed the Nanopore flow cell (MinION), a

miniaturised sample chamber incorporated into a USB device that is plugged directly into a computer for output and analysis, circumventing the need for a separate sequencing machine. David also presented a case study where sequencing was used to distinguish sources of *Vibrio cholerae* from the Haitian outbreak.

Ken Forbes (Univ. Aberdeen) described a WGS project for *Campylobacter*, ultimately in order to assign source attribution to clinical cases. He used a variety of approaches including: 7-loci MLST; 53-locus rMLST; 'known' or 'defined' loci; all loci (*de novo* reciprocal best hit); SNP profiling. Subtyping techniques provided sufficient discrimination for source attribution. The question of how to define a 'species' was raised e.g. from SNP analysis. Ken indicated that there is a need to link phenotype & genotype, he also suggested that WGS could be used in real-time strain typing, to identify chains of transmission.

Tim Dallman (HPE) described a large sequencing project for STEC, (led by John Wain), where a pseudo-sequence of all of the SNP-based differences was created to visualise phylogenies. This was important for isolate tracking and when used with enhanced surveillance questionnaires highlighted previously unidentified links in outbreaks, e.g. in two different PT8 STEC outbreaks. Tim also compared WGS with other high resolution subtyping methods such as MLVA, which can be as sensitive as WGS, but is species-dependent. Also, inherent practical issues with MLVA mean that WGS allows for faster linking of isolates. SNP-based phylogenies have been correlated with major PT and nicely demonstrate recent evolution of STEC O157. Tim concluded that WGS has a role in identification; context of an outbreak and in risk assessment.

Ulrich Dobrindt (Univ. Muenster, Germany) spoke about extraintestinal *E. coli* and avian-associated *E. coli*, using sequencing based typing methods. These *E. coli* types have been implicated as zoonoses. He showed that WGS phylogenies tallied well with rMLST and that the correlation with 7-loci MLST was still reasonably good. However, higher resolution information was required to differentiate clonal groups. Virulence-associated markers were not sufficient to separate clinical isolates, but it was possible to separate avian pathogenic isolates from faecal isolates, based on WGS-based phylogenies. There is a need for identification of appropriate discriminative markers, based on WGS.

John Coia (SSSCDRL) gave a very detailed perspective from a reference laboratory point of view. He briefly summarised an EFSA-run symposia also looking at the role of WGS. John compared the pros and cons of traditional and new sequence-based genotyping methods and pointed out that although one of the main problems with WGS is in (skilled) interpretation and analysis, he felt that area was improving with automation. An outbreak of a rare *Salmonella enterica* serovar, *S.* Mikawasima was highlighted as a case study, showing that WGS out-performed PFGE typing which is the current convention. However, consideration of changes in sequencing technology, read-lengths, bioinformatics bottlenecks, analysis and quality assurance need to be taken into consideration. Also need to decide the level of discrimination that is required: i.e. SNP-based 'vs' defined gene-sets. In a public health setting, WGS needs to be fast, comprehensive and labs need to work as a network, which all requires investment.

Lesley Allison (SERL) also provided a reference laboratory perspective, focusing on STEC. She described a project sequencing 160 well characterised *E. coli* O157:H7 isolates, analysed by reference-based mapping and SNP detection; and de novo assembly for gene-by-gene analysis.

Sequencing-based analysis correlated well with the epidemiological data, case with < 3 SNPs were considered to be related, while those with > 8 SNPs were most likely not related. One particular genotype was significantly associated with severe disease. There were issues with in silico MLVA profiling because of the repeat regions, which meant that backwards compatibility was not so strong.

David GE Smith (Moredun Research Institute) provided data on paratuberculosis (Map) as an example of a multi-species and environmentally-transmitted pathogen. David noted that there is still some debate as to whether it is truly a zoonotic pathogen, but pointed out that genome-based approaches can be used to better examine transmission pathways and relationships between isolates from different origins. WGS was applied to 130 isolates from multiple host species and of global distribution, showing some clustering based on host origin, but no clear geographical differentiation. WGS did not correlate well with conventional typing methods, which may explain some of the previous confusion with isolate tracking. Cost is likely to be a major barrier in veterinary diagnostics and epidemiology, although sequencing-based methods will be far more discriminatory for future work on MAP.

Leighton Pritchard (James Hutton Institute) examined the strengths, weaknesses, opportunities and threats to utilisation of sequence-based analysis. He pointed out an important need to share methods and results (online) so that they can be reproduced. Although short-read approaches (e.g. Illumina) are stable and widespread, Leighton, like David Ussery, pointed out the advantages of new technologies, e.g. Nanopore. Lack of appropriate expertise, datasharing and reproducibility are all important areas to consider, as is poor experimental design for research questions. However, movements such as the Global Microbial Identifier program are a sensible way forward and should be able to overcome threats such as standardisation, skills and data curation.

Breakout groups

Jacqui McElhiney (FSA-S) introduced the breakout session, firstly by summarising the perspective from the Food Standards Agency, highlighting the example of the very large scale *Campylobacter* sequencing project and the recently initiated VTEC project. She encouraged sign-up to the collaborative effort of the Global Microbial Identifier project, for which FSA is in the steering group. She then asked four groups were asked to consider the main topics of:

1. Tracking Origins and Destinations
    - Population Genomics
    - Epidemiology
2. Polymorphisms and consequences for transmission/virulence
    - Tracking and tracing, e.g. clone selection
    - Comparative genomics
3. Resources and provisions
    - Diagnostics, typing and evolution
    - Reference lab perspective
    - Surveillance data sets
4. Bioinformatics Applications and Considerations
    - Bottle-necks
    - Data handling and management

Summary of breakout discussions

The main points that were raised are:

- Is sequencing the elixir to all our current public health issues?
- Does NGS have a particular place, e.g in outbreak tracking (i.e. 'reactive') or in routine isolate typing (i.e. proactive)?
- How do we move forward with NGS while conforming to legislation?
- Accreditation/quality control is essential
- Who decides what quality control / software / pipelines used?
- How will this be coordinated?
- How do we share (meta)data? And what metadata can be shared?
- How do we engage industry?
- Can we afford it and who pays?
- We have common issues so can we do it together?
- Can we better coordinate and maximise outcomes?
- Is Scotland / UK and its different public facilities / labs small enough to coordinate efforts?
- 'Scotland central sequencing facility' – Clinical, Agri-food (animal / plants), water, environment?

Appendix

Programme
Day One – 11th November 2014

Introduction and Session 1: invited presentations
Overview: Typing & Epidemiology using WGS

- David Ussery (Center for Genomic Epidemiology) "Third Generation Sequencing for Rapid Biosurveillance"

Tracking Origins & Destinations

- Ken Forbes (Aberdeen) "*Campylobacter* – layers of complexities"
- Tim Dallman (HPE) "Whole genome sequencing for National Surveillance of Shiga Toxin Producing *Escherichia coli* O157:H7"

Polymorphisms & Consequences for Transmission or Virulence

- Ulrich Dobrindt (Muenster) "Avian pathogenic *E. coli* and human extraintestinal pathogenic *E.coli* - is there a zoonotic risk or just an exchange of virulence and resistance genes?"

Session 2: invited presentations continued
Resources & Provisions

- John Coia (SSSCDRL) "Whole genome sequencing in the reference laboratory: the good, the bad and ugly"
- Lesley Allison (SERL) "Our experience of NGS at SERL"

Veterinary tracking

- David GE Smith (Moredun) "Adding a "One Health" perspective to pathogen NGS"

Applications and considerations

- Leighton Pritchard (Hutton) "Sequencing and Beyond"

Session 3: Posters and wine reception

Day Two – 12th November 2014

Session 4: Breakout sessions

- Jacqui McElhiney (FSA-S) "Introduction to breakout sessions"

Topics to include:
Tracking origins & destinations

- Population genomics
- Epidemiology

Polymorphisms & consequences for transmission / virulence

- Tracking and tracing, e.g. clone selection
- Comparative genomics

Resources & provisions

- Diagnosis, typing and evolution
- Reference lab perspective

Bioinformatics Applications & Considerations

- Bottle-necks
- Data handling and management

Session 5: Offered papers

Session 6: Feedback from breakouts:
Summary of the discussion groups

- Ian Toth (Hutton)

Collation & summary of the meeting

- David Smith (Moredun)

Breakout sessions: Individual group discussions

Group 1

Surveillance

- Live or dead? (RNA vs DNA?) – does sequencing tell us what we need to know?
- Metagenomics vs culture ('culture' is the gold standard in legislation)
- Following or influencing legislation?
- Standardisation of methods vs adoption of new methods
- Is sequencing useful for all aspects of public health or limited to certain areas? – e.g. rapid response to outbreaks
- How do we engage industry and surveillance?
- Privileged information, business size and in-house capacity etc.

Infrastructure / expertise needs

- NGS – essential for effective typing/traceability if cost effective!
- Attribution of causative strain(s) in mixed samples (e.g. metagenomics)
- LGC, PHE, FERA
- – no service support capacity on sequencing
- QA accreditation
- Common vocabulary for recording sample ID etc., e.g. standardised questionnaire
- Agreed criteria for sharing metadata vs "semi-confidential"
- FOI
- "Ambulance chasers"
- Sequence data
- How long retained; How long confidential; How useful without isolate
- Storage – Cloud? Secure?
- Raw reads vs Bam file processed data
- Data standardisation – internal or industry standard

Data analysis

- Bioinformaticians!!!!
- Training?; who?; how many? What capabilities
- Analysis needs depends on purpose
- Reference lab; research lab; hospital lab
- Communication lab – agency/authority
- When interlink? Public health/reference lab with research
- NEED
    - Rapid ID; sufficient level of typing; depends on pathogen; speed/accuracy
    - Fitness for purpose – does it need WGS?

Whole chain approach

- How funded?
- Common goal – agency agenda
- WHO PAYS?

Group 2

Routine surveillance

- Tracking (cycling, environment)
- Prioritise; Several needs / agencies – joined up or not? (affects central source provision)
- Focus has been on 'end of chain' – infection
- What level of information is required? Is MLST enough?
- Controls and standards – robust enough for legal presentation?
- No. of samples:
    - survey-based 1000s (~4000)
    - Local authority budgets, cost an issue
    - Not discovery – know what we're looking for
- Quantification may be important, not just ID
- Sequencing good for discovery and precise tracking but this not always the focus
- Cost equivalence to existing tests (economic pressure vs gain of information)
- Imbalance of information depending on region / area budget
- Historical samples at reference labs, not necessarily elsewhere; academic / isolate collection sample bias.

Lab provision /expertise

- Not enough on individual level
- Matching expertise to location
- PHE - Regional front line – epidemiology centre
- Central NHS – strategic roll-out easier than Scotland
- Regional labs – local authorities (local funding): questionnaires/surveys may differ - less coordination? Duplication?
- FSA-UK vs FSA-S – provides regional level funding – currently similar organisation but may differ in strategy for next year.
- Central, commercial provision may be preferable (rolling funding, tech ages, etc)

- Accreditation/certification for what wet work doesn't cross over to sequencing and data analysis (possible to FAST output steps?)

Data intelligence sharing

- Existing databases of typing etc at FSA and LA
- Establish standards for metadata (confidence in this data)
- Anonymisation and restricted subsets necessary for sharing (restricted access possible) – policy/privacy issues
    - E.g. private water supply data / clinical samples /food production (process / retail)
- PHE reads to ENA/SRA: date of isolation; region, clinical, animal, food; patient data private; detailed release for collaboration on ad hoc basis
- UK pretty good for data release
- Requirement to release supporting data
- Academic data release policies emphasise sharing
- ID doesn't necessarily need contextualisation for downstream; public data feeds in here – more back and forth if ref lab helps
- What is quality of public data? Accreditation /certification – correction mechanism
- Release schedule: academia (pressure to publish) vs public health (solve immediate problems and horizon scan)

Data storage

- Sequencing: central resource: ENA/SRA, NCBI, DDBJ (assembly/annotation standards)
- Derived data – more case by case
- Focus
- sequences: BIGSdb, pubMLST
- Organism-based: e.g. pseudomonas.org/FlyMine etc (need both)
- Disparate data based unstable: funding/academic life cycle
- Take some resources under central public funding for stability, where relevant to public health – bureaucracy / clear statement of purpose
- Data exchange and storage standards
- Visualisation:
- Are scientists best at this?
- Advertising / graphic design / HCI?

Making links

- Workshops / groups
- Common problems (big data sequencing) - common solutions (common funding / efficacy
- SEPA: Sequencing
- IFFN: Food fraud: needs seq?
- water: indicator organisms: E. coli VTEC burden (ag / animals)
- Public health – food – ag – environment (fairly good chains of communication)
- Some oversights (SEPA in outbreak, e.g. difference s in sampling programmes - opportunities for joining up better
- Common sequencing databases – common sampling and strategy needed

Group 3

Gaps

- Screening
- Rapid methods for detection of pathogens - isolates for sequencing
- Clinical-food-water-veterinary
- Seasonality
- More work needed to identify sources
- Combine epidemiological data with microbiology and knowledge of production chain to identify risk factor

Surveillance

- Source is the animal
- Strategy for sampling faeces at abattoir
- Rapid methods for multiple pathogens
- Isolates for sequencing
- Would help to target testing strategies further up the chain
- Identify biggest risks in food chain to target sampling and sequencing ie how are pathogens getting into food chain?
- Middle of chain LA samples – sequenced
- Joined up approach
- Strategy for sampling at each point in the chain
- Identify what point you need to start
- Different for each pathogen

Lab provision and expertise

- Standardised platforms; QA/QC
- Bioinformatics a major gap; needs to work towards an automated standardised pipeline; future aim
- Scotland centralised facility
- 'Scottish food, water and environment lab'
- 1000 samples annually
- Sequenced using same pipeline as clinicals
- Controlled access to metadata: food (official control); clinical (NHS)
- Stratify metadata according to confidentiality
- Need training for ref and public health labs on bioinformatics to help understand data coming out of central sequencing facility
- Opens up access to research
- Strengthens grant proposals
- Industry – need to think how we open system to encourage them to submit isolates – data confidentiality – demonstrate benefits
- Environmental sampling - JHI and others, e.g. isolates from research and monitoring on soils and plants also to go to facility for typing, e.g. environmental E. coli

Group 4

Source attribution

- Relevance – required information
- New emerging pathogens, e.g. O104:H4
- Baseline data, quality of reference strains
- 'Under-studied' organisms
- Microbial ecology

Standardisation of data:

- analysis processing, e.g. MIAME (arrays); parameters/protocols;
- quality insurance (thresholds/foreign DNA); communication platforms
- Infrastructure
- Service quality; Computational complexity; Bioinformatics skills; Professional expertise; public lab – network of experts (avoid redundancy)

Links and networks

- Between facilities
- Strategies for data mining, sharing, protection
- Guidelines
- Legal protection ,intellectual property
- Meta-studies on same data sets
- Raw data vs metadata
- Legislation; international scale
- Links to industry
- Clients; cooperations
- Training and education
- Improved communication
- Exchange/ data integration
- Collaboration with other disciplines
- Funding bodies
- Responsibilities
- Closer cooperation – "One Health"
- Politics? Governmental bodies HPE/FSA etc.

Abstracts

Poster and offered presentation abstracts

Rodrigo Bacigalupe

The Roslin Institute

Title: Evolution of *Staphylococcus aureus* after a human to livestock host-jump event

Authors and affiliations: *Rodrigo Bacigalupe[1], María A. Tormo[2], José Penades[3] and J. Ross Fitzgerald[1].*

[1]The Roslin Institute, University of Edinburgh, Scotland, UK; [2]Centro de Investigación y Tecnología Animal, Instituto Valenciano de Investigaciones Agrarias, Segorbe, Castellón, Spain; [3]Institute of Infection, Immunity and Inflammation, University of Glasgow, Glasgow, Scotland, UK.

Main text

Staphylocccus aureus is a major human and animal pathogen. Most strains infect a single host species, but transmission between humans and livestock in both directions are increasingly being reported, consistent with the zoonotic potential of *S. aureus* and its threat to public health. Some zoonotic episodes may lead to pathogen host-adaptive changes that result in successful host-switches, but the underlying molecular mechanisms are still poorly understood. Here, in order to understand the adaptive evolution of *S. aureus* after a zoonotic transmission, we simulated host-jumps by performing experimental intra-mammary infections of sheep with *S. aureus* strains of human origin. To simulate onward transmission to other sheep, serial passages of S. aureus were made in multiple sheep for up to 14 months. We performed whole-genome sequencing of progenitor and *in vivo* passaged isolates and compared them using an array of bioinformatic tools to identify accumulated genomic changes, revealing mutations in genes involved in transport, virulence, regulation and metabolism. Co-infection experiments with original and passaged strains revealed that some of the accumulated mutations contributed to enhanced fitness during intra-mammary infection. Phylogenetic analysis based on core genome SNPs enabled us to track the evolutionary trajectory of the passaged strains revealing the existence of population bottlenecks associated with transmission and establishment of infection in new hosts. Overall these data provide new information relating to the critical early events associated with host-adaptation and within-host diversification.

Bernhard Merget

University of Aberdeen, James Hutton Institute

Title: The effect of diverse genotypes on environmental adaptation of *Escherichia coli*

Authors and affiliations Bernhard Merget[1, 4], Norval Strachan[1], Ken Forbes[2], Fiona Brennan[3], Nicola Holden[4]

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Main text

Escherichia coli belong to the group of gastrointestinal-associated bacteria with its main reservoir in endothermic animals. The genetic variability within this species is very diverse and while most serotypes are harmless to animals, a few are pathogenic, transmitted through the food chain or by direct contact. These serotypes and their host adaptation are well described for multiple animal host species. However, *E. coli* can become persistent in the wider environment, including in plants, water and soil. Substantial differences in environmental parameters require wholescale adaptive changes

for *E. coli*, which may be short term to allow transient colonisation or long-term for more persistent growth and survival.
In this project phenotypes for environmental adaptation, including growth or motility were assessed in genetically diverse *E. coli* isolates. The hypothesis is that the origin of the strain and therefore the adaptation to environmental parameters has a great impact on the ability to host adaptation. Correlation of genotype with adaptive phenotypes could help to assign risk criteria in the context of food security.

Anne Holmes

NHS Lothian

Title Comparison of Next-Generation Sequencing with traditional methods for outbreak detection and epidemiological surveillance of *Escherichia coli* O157

Authors and affiliations

Anne Holmes[1], Lesley Allison[1], Melissa Ward[2], Richard Clark[3], Angie Fawkes[3], Lee Murphy[3], Mary Hanson[1]

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Main text

We have compared our routine typing methods (phage typing and MLVA) with next-generation sequencing (NGS) for genotyping *E. coli* O157. One hundred and six *E. coli* O157 strains isolated over a five year period from human faecal samples in Lothian, Scotland were analysed. These included 10 cases from a UK-wide outbreak and 10 other epidemiologically-linked cases. Isolates were sequenced using the Ion Torrent Personal Genome Machine and analysed using a reference-based assembly approach with the Sakai strain. A total of 8708 core genome SNPs were identified among the 106 isolates, although 47% of these were found in only 6 'atypical' *E. coli* O157. Phylogenetic analyses showed the SNP data correlated well with phage type, MLVA and the epidemiological data. Epidemiological links existed between cases whose isolates differed by 2 or fewer SNPs and not among cases whose isolates differed by >8 SNPs; whilst isolates differing by 3 – 8 SNPs were indeterminate. This information could support national surveillance of VTEC infection in Scotland and outbreak detection. NGS did not identify any additional putative links in the dataset compared with MLVA; however in two cases confirmed MLVA single locus variants were genetically divergent. Furthermore, NGS produced a more accurate phylogeny compared with MLVA suggesting core genome SNP data is more suitable for epidemiological surveillance of *E. coli* O157. Together the results show NGS provides a high resolution subtyping method, with superior phylogenetic accuracy compared with MLVA. However, the method is less convenient than MLVA, and issues relating to ease of performance and standardisation, as well as IT infrastructure and data storage need to be addressed before it is used routinely.

Bruno S Lopes

University of Aberdeen

Title: A longitudinal study of interventions and Campylobacter genotypes from broiler farms across Northern Ireland***.***

Authors and affiliation: Bruno S. Lopes[1], Norval J. C. Strachan[2], Fraser Whyte[3], Marion Macrae[1], Ann Thomson[1], Meenakshi Ramjee[1], Nick Sparks[3] and Ken J. Forbes[1]

Main text

Objectives: To determine the efficacy of broiler house biosecurity interventions (biosecurity barrier, acid treatment of drinking water and flyscreen). To utilise genotyping data to detect carry over between crops, identify potential source of flock colonisation and how genotypes change during crop lifetime.

Methods: 24 Broiler farms were sampled pre-thin and at clearance for 8 crops. Campylobacter was isolated, and whole genome sequenced. MLST genotypes were obtained.

Results: Prevalence was higher ($P<0.0001$)at clear 79.9% than pre-thin 47.1%. No significant differences were found in prevalence for intervention compared with control farms ($P>0.05$). At-clear 6 farms were always positive ($P=0.005$). Since interventions had no effect all farms were combined for genotyping analysis.

There were 64 flocks, out of a possible 168, that were positive at clear and also positive pre-thin in the following crop. Of these 64 flocks 6 had the same genotype at clear and at following pre-thin and this is higher than expected by chance ($P=0.04$). Of the 6 positive flocks, it is noticeable that two farms have two carry overs each ($P=0.009$). However, carry over of the same genotype only explains 4% of overall flock positivity prevalence.

STRUCTURE inferred putative source pre-thin as cattle (0.315), sheep (0.217), wild birds (0.381) and pigs (0.086). Two sequence types (ST814 and ST257) were more common at clear and flocks were more likely to change from C. jejuni to C. coli ($P=0.03$).

Conclusion: The interventions were ineffective, carry over between flocks explains only a small amount of positivity, some farms are continually positive and a number of external source reservoirs contribute to flock prevalence.

S. Chipchakova

University of Aberdeen

Title: Addressing food security by controlling the risk of food poisoning: a case study of listeriosis in the Scottish smoked salmon industry***.***

Authors and affiliation: Prof. N. Strachan, Dr Ken Forbes, Dr D. Watts and Dr F. Perez-Reche
University of Aberdeen, School of Biological Sciences

Main text

L. monocytogenes is a food-borne pathogen causing listeriosis (≈180 cases/year in the UK) mainly in vulnerable groups (e.g. pregnant, elderly people) with mortality of up to 30%. Ready-to-eat foods (such as smoked salmon) are a potential source of contamination (e.g. FSA survey showed that 17% of cold smoked fish contained *L. monocytogenes* but none were above the legal limit of 100 CFU/g). The aim of this project is to understand the sources of *Listeria* in salmon products by subtyping and comparing samples from fish farms, smoking plants and clinical isolates, as well as to assess the risk of contamination. This data will be integrated together by modelling pathogen's growth along the processing chain.

List of participants

Forename	Surname	Organisation
Alison	Aitken	Glasgow City Council
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Kerry	Bailey	City of Edinburgh Council
Robert	Beattie	City of Edinburgh Council
Fiona	Brennan	The James Hutton Institute
Kaye	Burgess	Teagasc, Ireland
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Tim	Dallman	PHE
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Mary	Hanson	Royal Infirmary of Edinburgh
Nicola	Holden	James Hutton
Anne	Holmes	NHS Lothian
Marianne	James	Food Standards Agency
Bruno S	Lopes	University of Aberdeen
Nadejda	Lupolova	Roslin Institute
Jacqui	McElhiney	Food Standards Agency
Bernhard	Merget	The James Hutton Institute
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Meenakshi	Ramjee	University of Aberdeen
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David	Ussery	Oak Ridge National Laboratory, USA
Adam	Wyness	The James Hutton Institute

Glossary of acronyms

FSA	Food Standards Agency
FSA-S	Food Standards Agency in Scotland
HPE	Health Protection England
HPS	Health Protection Scotland
JHI	James Hutton Institute
MRI	Moredun Research Institute
MRP	main research provider
SERL	Scottish *E. coli* O157/VTEC Reference Laboratory
SSSCDRL	Scottish *Salmonella*, *Shigella* and *Clostridium difficile* Reference Laboratory