



Public Health
England

Application of whole genome sequencing for public health interventions around foodborne pathogens

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Estimates of foodborne infectious disease



- Estimated more than 500,000 cases of foodborne illness linked to known pathogens in the UK annually
- High economic burden - £1.8 billion annually in medical, financial and welfare costs

Estimate of number of cases per year of illness due to common foodborne pathogens

<i>Campylobacter</i>	280,000	<i>Cryptosporidium</i>	2770
<i>C. perfringens</i>	80,000	<i>Giardia</i>	8000
<i>E. coli</i> O157	10,000	<i>Norovirus</i>	74,100
<i>Listeria</i>	183		
<i>Salmonella</i>	33,100		

O'Brien et al, BMJ Open 2016
doi: 10.1136/bmjopen-2016-011119.



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Gastrointestinal Bacteria Reference Unit

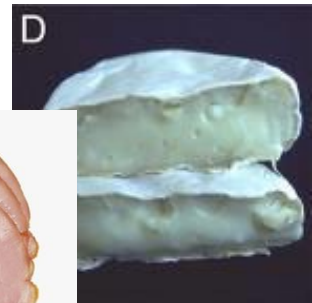
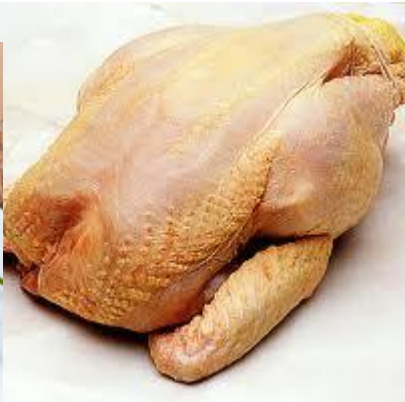
- National Reference Laboratory
- *Salmonella* Reference Service
- *E. coli*, Shigella, Vibrio and Yersinia Reference Service
- Foodborne Pathogen Reference Service – *Listeria monocytogenes*, *Bacillus cereus*, *Clostridium botulinum* and *Clostridium perfringens*, *Staphylococcus aureus*
- *Campylobacter* and *Helicobacter* Reference Service

Receive bacterial isolates from clinical microbiology laboratories and food and water laboratories ie from clinical cases and from foods, across England and Wales for identification and typing for national surveillance and for outbreak detection and investigation



Value of Typing

- Provides information to assist the understanding and management of local outbreaks/incidents
- Identifies clusters and outbreaks occurring at local, regional, national level
- Provides microbiological evidence to identify and track source of contamination so effective control and prevention measures can be implemented
- National surveillance and trend monitoring over time
- Monitoring effectiveness of control and preventative measures





Outbreak investigation questions

- Is there an increase in a particular type – a cluster of cases/outbreak?
- Is the type from clinical cases same as the type from food – if available?
- Have we seen this type before in food?
- Is this a known type in the UK or elsewhere?



Move to WGS

- WGS provides a single one step method for identification and typing and provides a wealth of additional information
- Faster TATs, lower costs
- High quality data, standardisation, rapid global comparability
- Safety



- Highest degree of resolution for strain discrimination
- Provides phylogenetic information showing unequivocal strain relatedness
- Improved cluster detection
- Provides information on potential sources and geographical location
- Ability to rapidly screen large number of isolates for virulence genes, AMR genes
- Leads to improved control measures

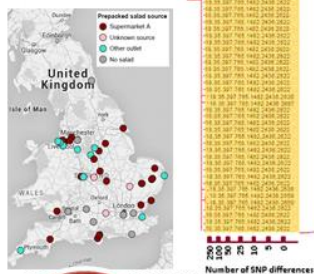
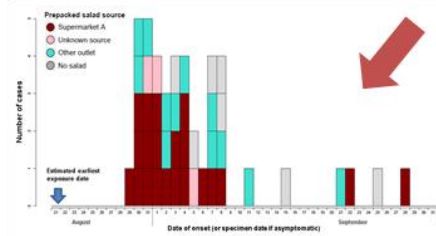


Overview

WGS of:
Salmonella
E. coli STEC
Campylobacter
Listeria

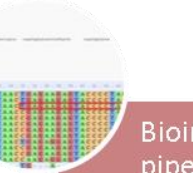
Cluster 15:2378
1 isolates within search criteria, 3 isolates in the database

SNP Address	Molis ID	Exit ID	Sending Lab	PHE Region	PHE Centre	HPT	Country	Sample Type	Isolate Info	NHS Number	First Name	Surname	Date of birth	Post Code	Receipt Date	Sample Date	Report Date	Foreign Travel	Organism Identified
2.154.315.2041.2320.2378.3088	H160280283	16.0900451	DX6780100-HPA WEST MIDLANDS	Midlands and East of England	West Midlands	West Midlands East	England	Human Faeces							2016-01-15	2016-01-07	2016-01-19	Morocco	Escherichia coli
2.154.315.2041.2320.2378.2814	H153980184	15-2077330-1	DX6120200-HPA S.WEST BRISTOL	South of England	South West	Avon, Gloucestershire and Wiltshire	England	Human Faeces							2015-09-25	2015-09-18	2015-10-06	Morocco	Escherichia coli
2.154.315.2041.2320.2378.2546	H152920519	M.15.1798871	WHISTON HOSPITAL L35	North of England	North West	Cheshire and Merseyside	England	Human Faeces							2015-07-14	2015-07-09	2015-07-16	Morocco	Escherichia coli



Outbreak cases

SNP Address	Number of SNP differences
H152920519	0
H153980184	1
H160280283	2
H123728471	3
H123828282	4
H123828118	5
H123828101	6
H123828102	7
H123828103	8
H123828104	9
H123828105	10
H123828106	11
H123828107	12
H123828108	13
H123828109	14
H123828110	15
H123828111	16
H123828112	17
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H123828189	94
H123828190	95
H123828191	96
H123828192	97
H123828193	98
H123828194	99
H123828195	100



SNP address on GDW

Bioinformatics pipeline

Sequencing

Sub-culture and extraction

- Identification
- Serotype
- MLST ST, CC
- 1 - 5 days → Virulence and AMR gene detection
- SNP typing - address
- Cluster detection
- Patient and food isolate matching

1 - 2 days

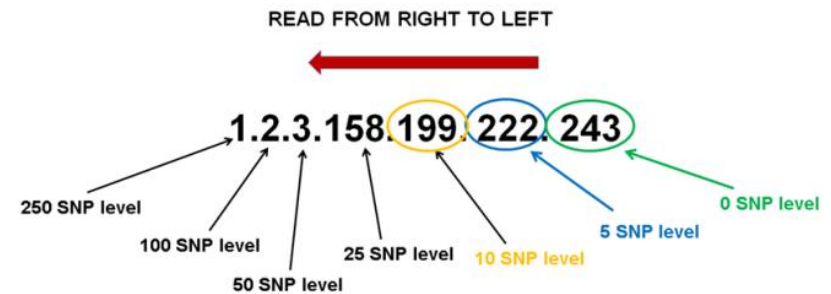
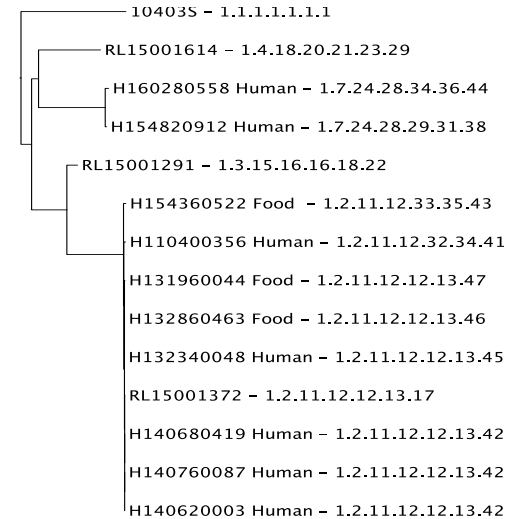
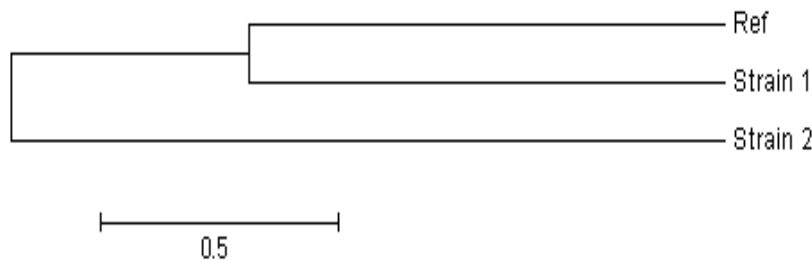
4 - 7 days



Assessing similarity between genomes by SNP typing

Single nucleotide polymorphism (SNP)
i.e. a different nucleotide to the reference genome

Ref	G	T	A	G
Strain 1	G	G	A	A
Strain 2	A	G	G	G



SNP address



WGS led surveillance

- Identify linked strains with unprecedented sensitivity and specificity
- Provides context for strains
 - Infer strain source
 - Native / Imported
 - Geographical signals within UK
 - Virulence potential
 - Demonstrate relatedness of isolates over years

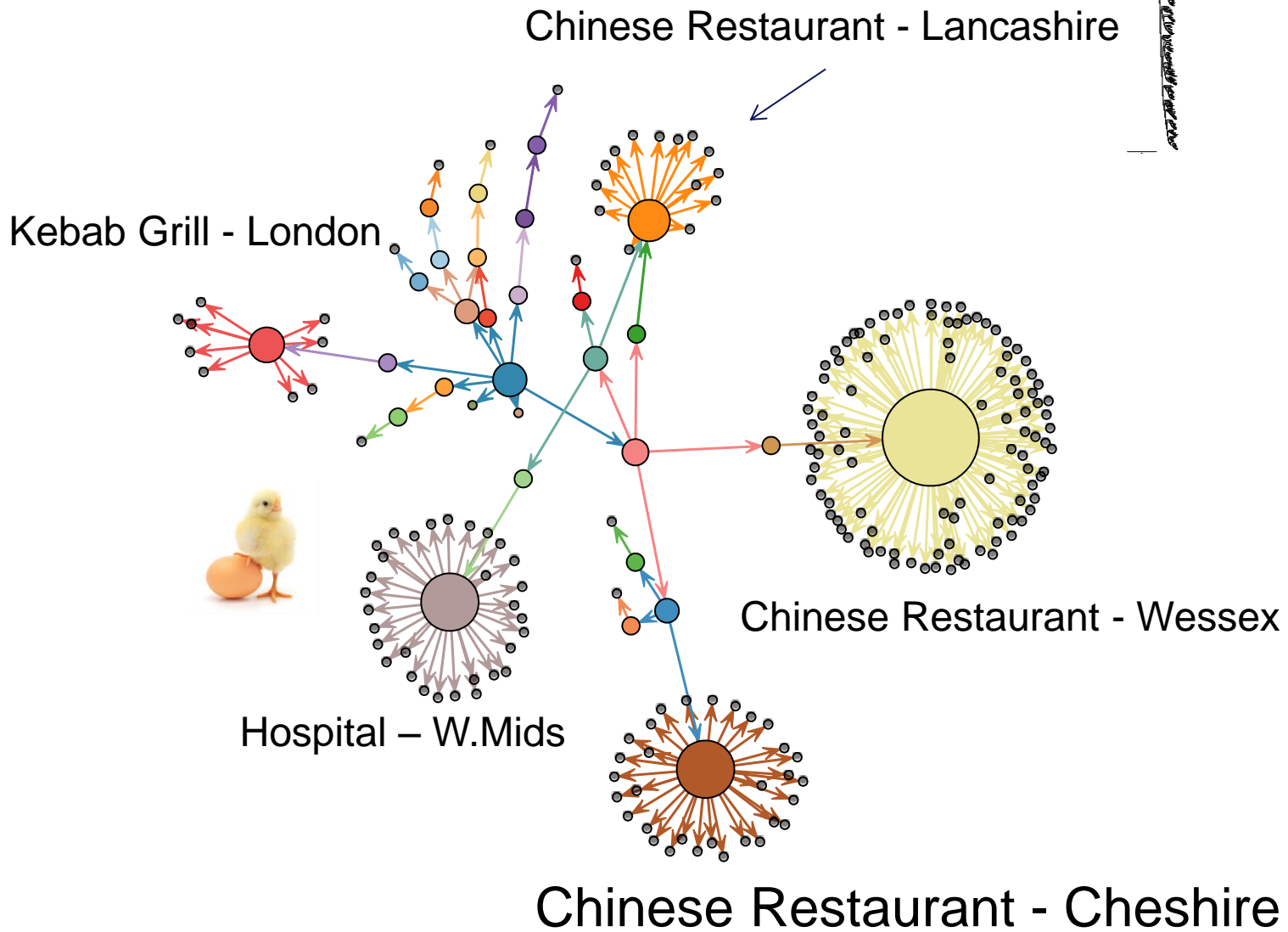
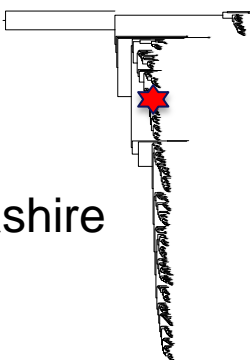
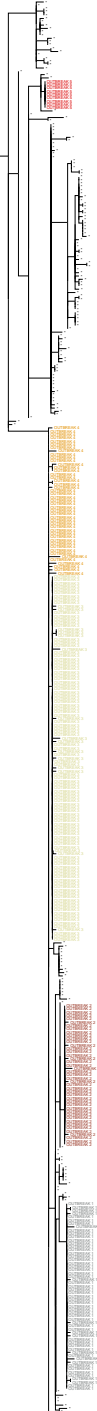
Building global databases – clinical, animal, food

NCBI BioProject PRJNA248042



International Outbreak

- Cases in Austria, Germany, France and Luxemburg plus French egg isolate with German egg mark – same MLVA or SLV as UK
- Trace back in UK – All outbreaks involved eggs from same German supplier
- Trace back investigations lead to sampling of egg production premises in Germany
- Company has 4 separate egg production plants supplied with layer flocks from single supplier
- European isolates sequenced – cases / eggs





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Salmonella Enteritidis

14b

Orange – Austria

Blue - France

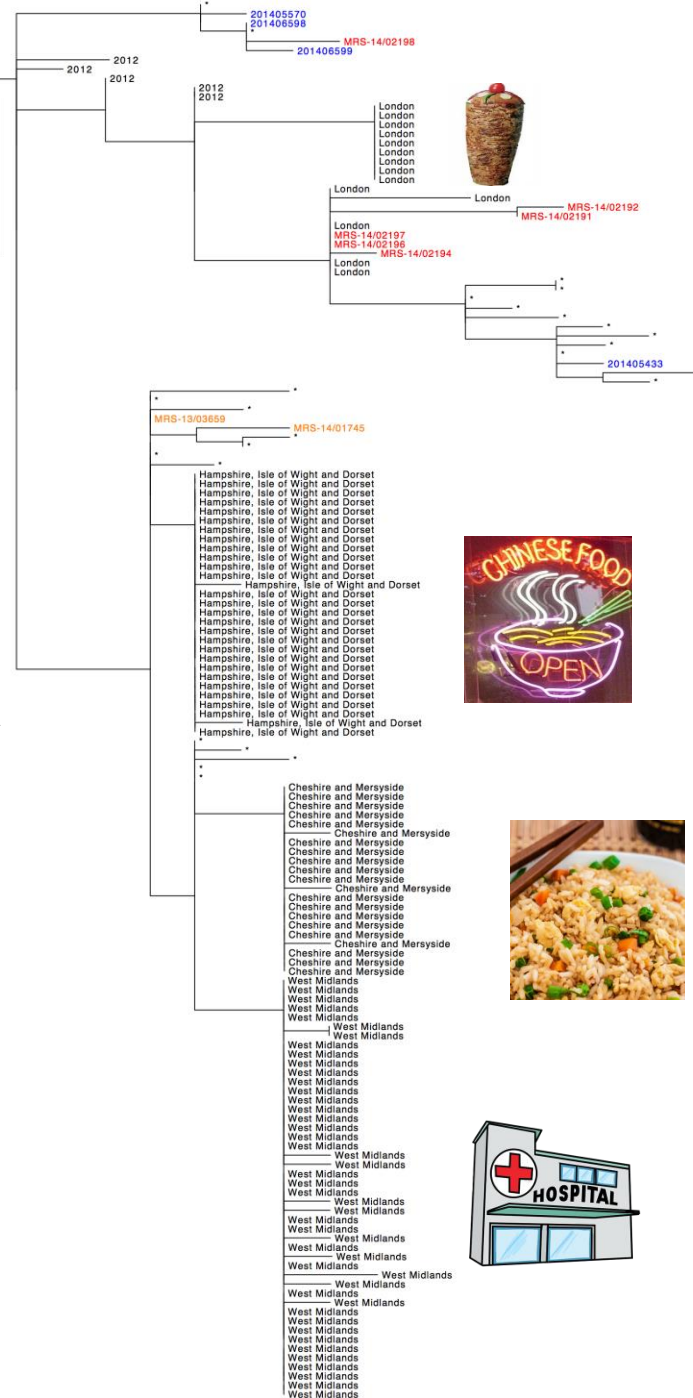
Red - Germany

Black - UK

1 12 environmental + egg isolates from site A
cases from Germany
2 cases from France

2 2 egg isolates from site B
7 cases from Germany
4 cases from France with trace back to B
4 Austrian and 1 Luxembourg case
32 England – 9 OB at kebab restaurant

3 Majority of UK isolates
4 cases from Germany
2 cases from Austria



1

2

3



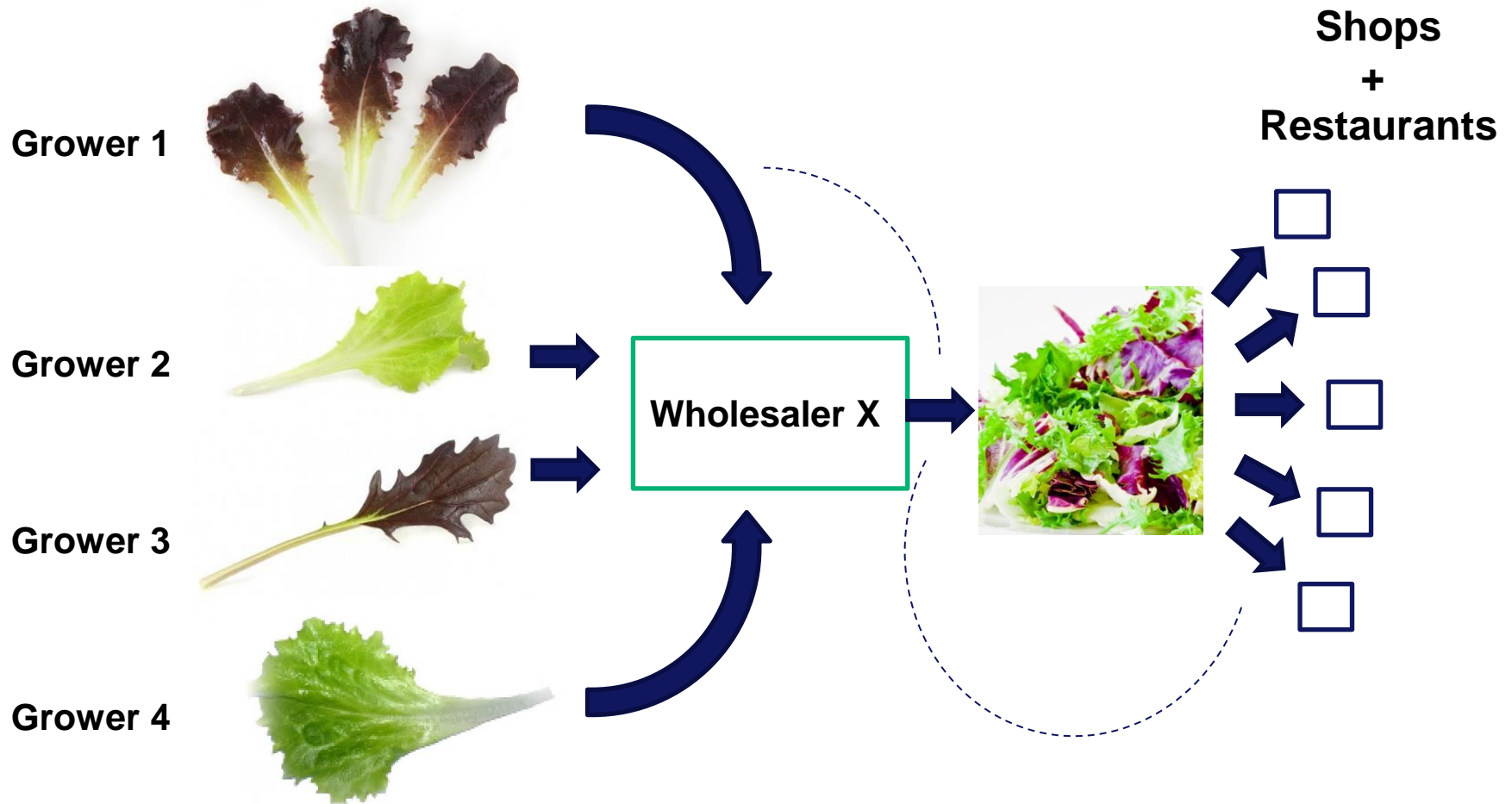
Shiga toxin-producing (STEC) *E. coli* O157

- Severe bloody diarrhoea and Haemolytic Uraemic Syndrome (HUS). Long-term renal, cardiac and neurological sequelae
- Limited treatment options – palliative care only as antibiotics contraindicated
- Zoonotic reservoir in the UK mainly cattle, sheep and goats, but almost all animals can act as secondary transmission vectors
- Transmission can be food or waterborne, via direct contact with animals or their environment, or person to person spread





Salad outbreaks are tricky!

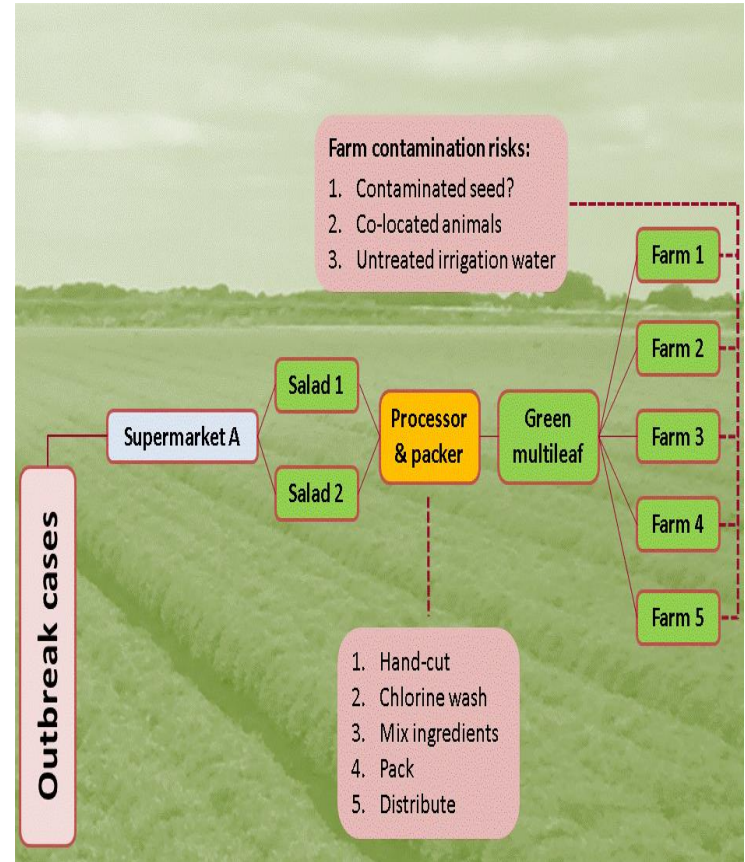


different salad leaf mixes on different days



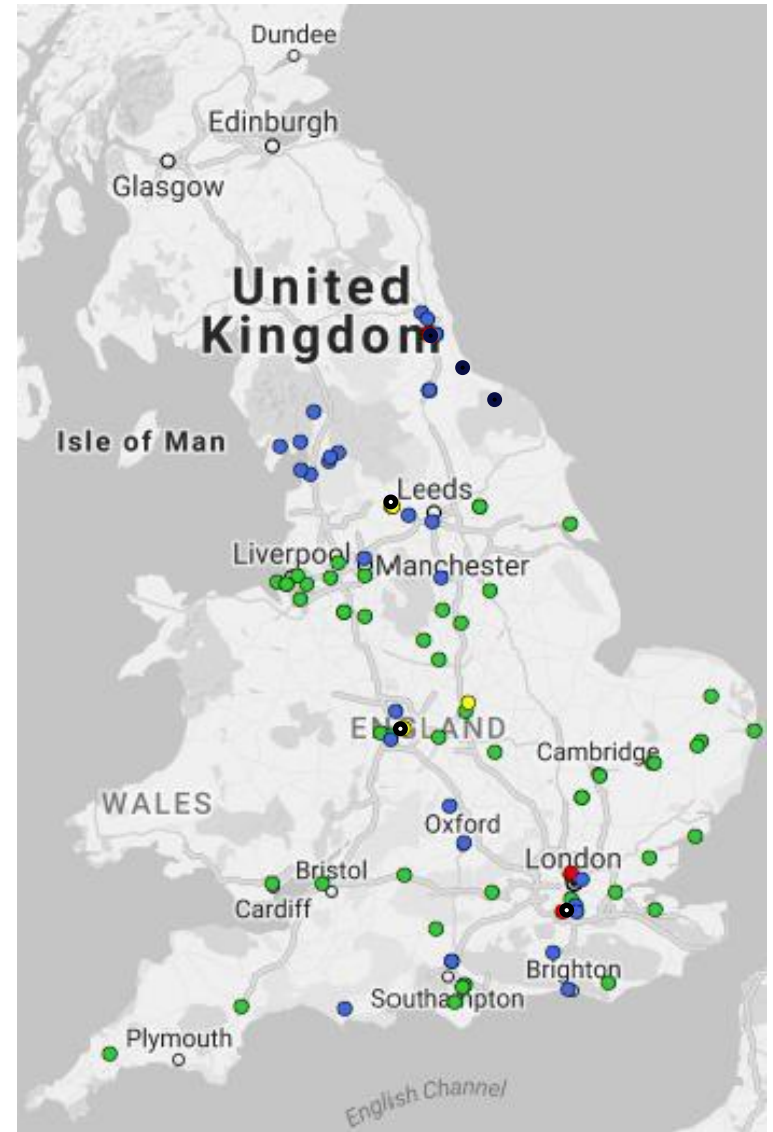
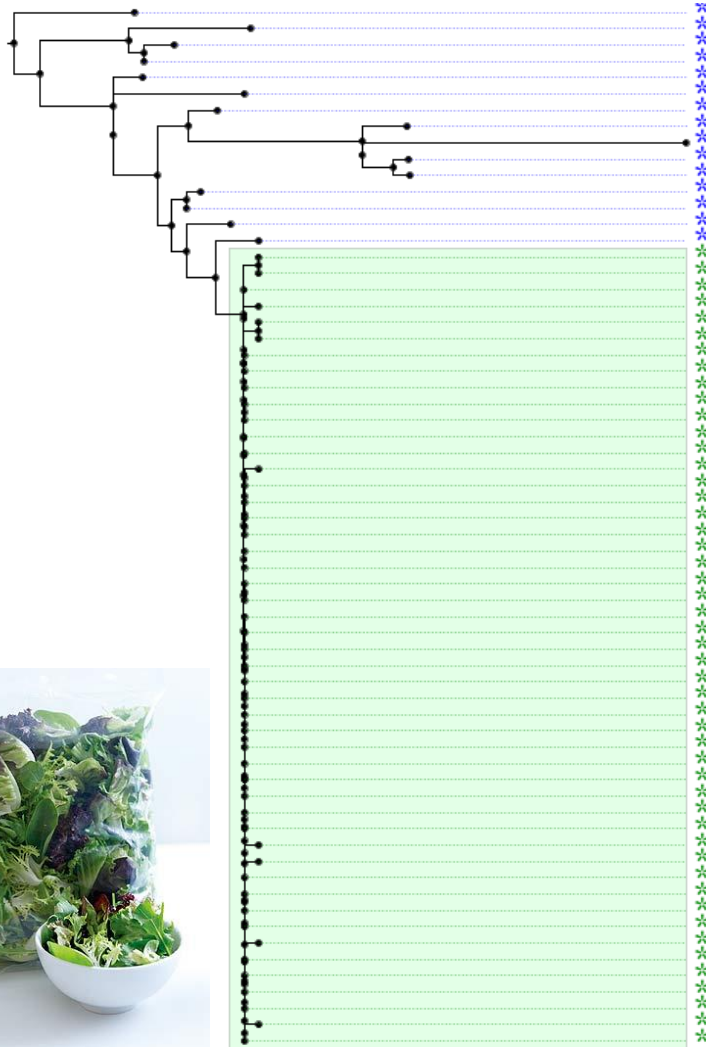
Deeper phylogenetic relationships : National context

- August 2015 – 40 cases of STEC O157 PT 8 detected following routine analysis of the WGS data. Not travel associated, but genetically close to UK cattle and environmental isolates in DB
- Cases exposed to prepackaged salads subsequently found to have particular type of salad leaf in common.
- Green multi-leaf lettuce, supplied to Supermarket A, was grown in England on five different farms, all samples -ve
- EHOs and local HPTs reported poor biosecurity on all farms, eg. animal ingress (wildlife) and use of untreated irrigation water from rivers and ponds



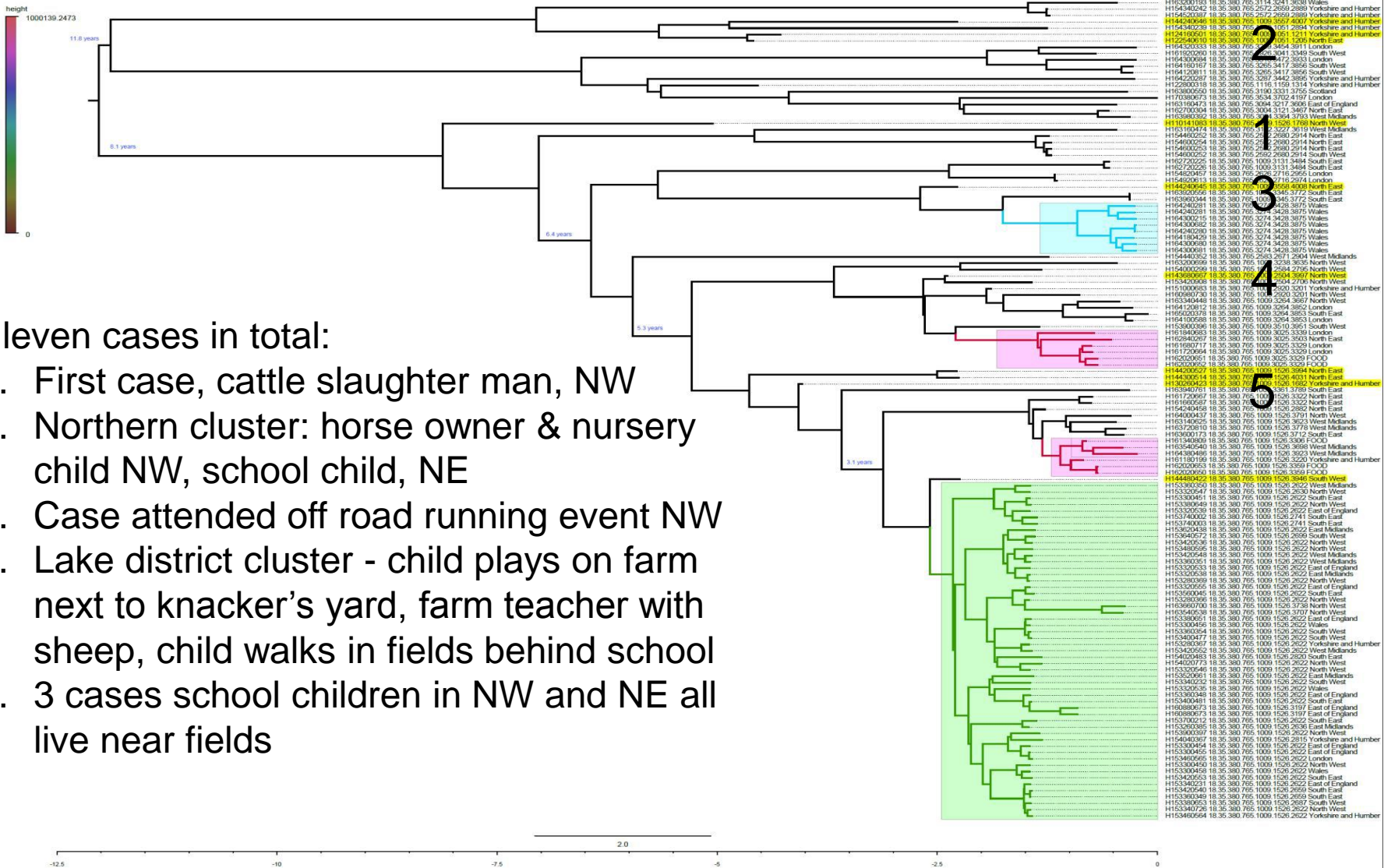


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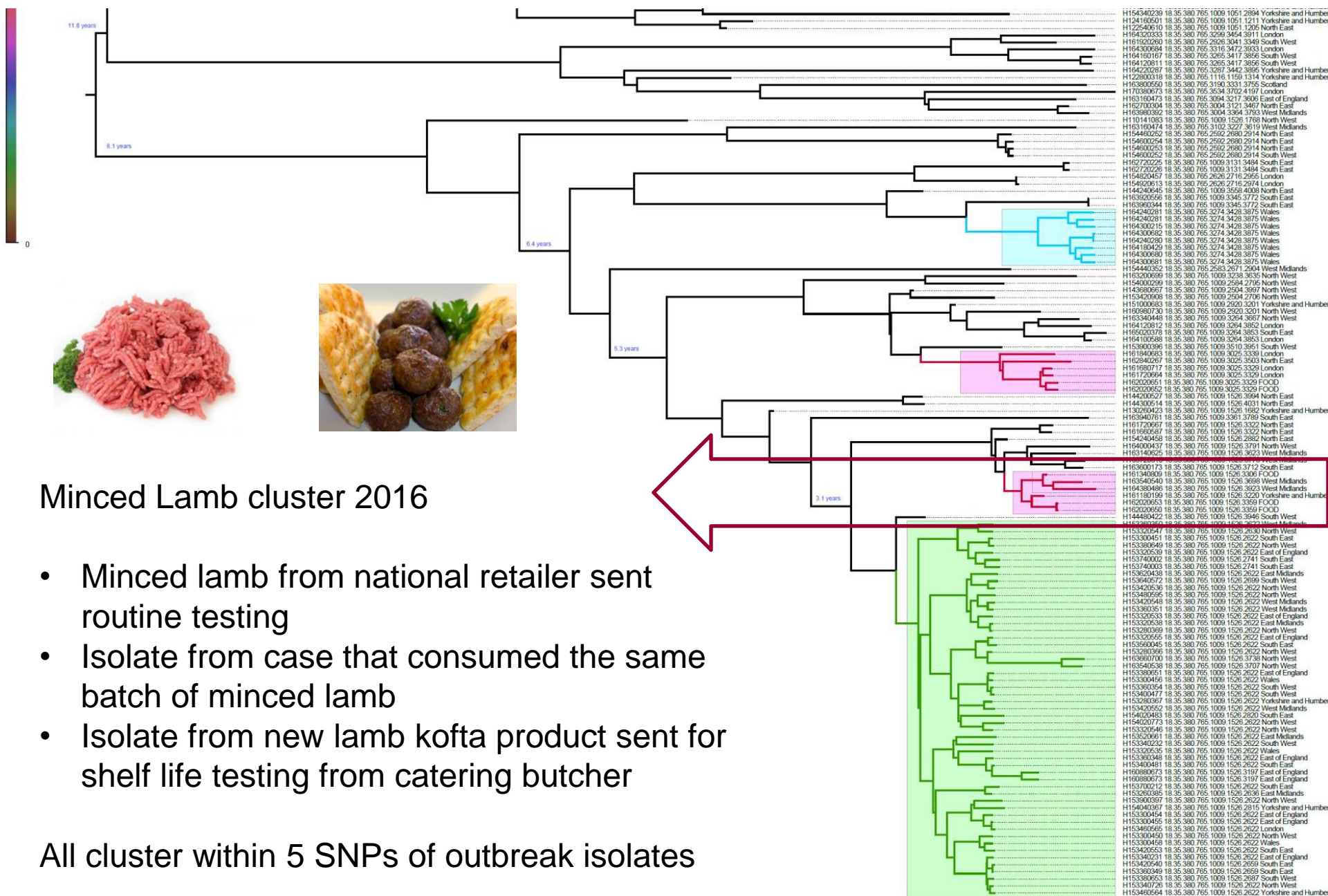


Pre-outbreak cases, 2012 - 2015:



Eleven cases in total:

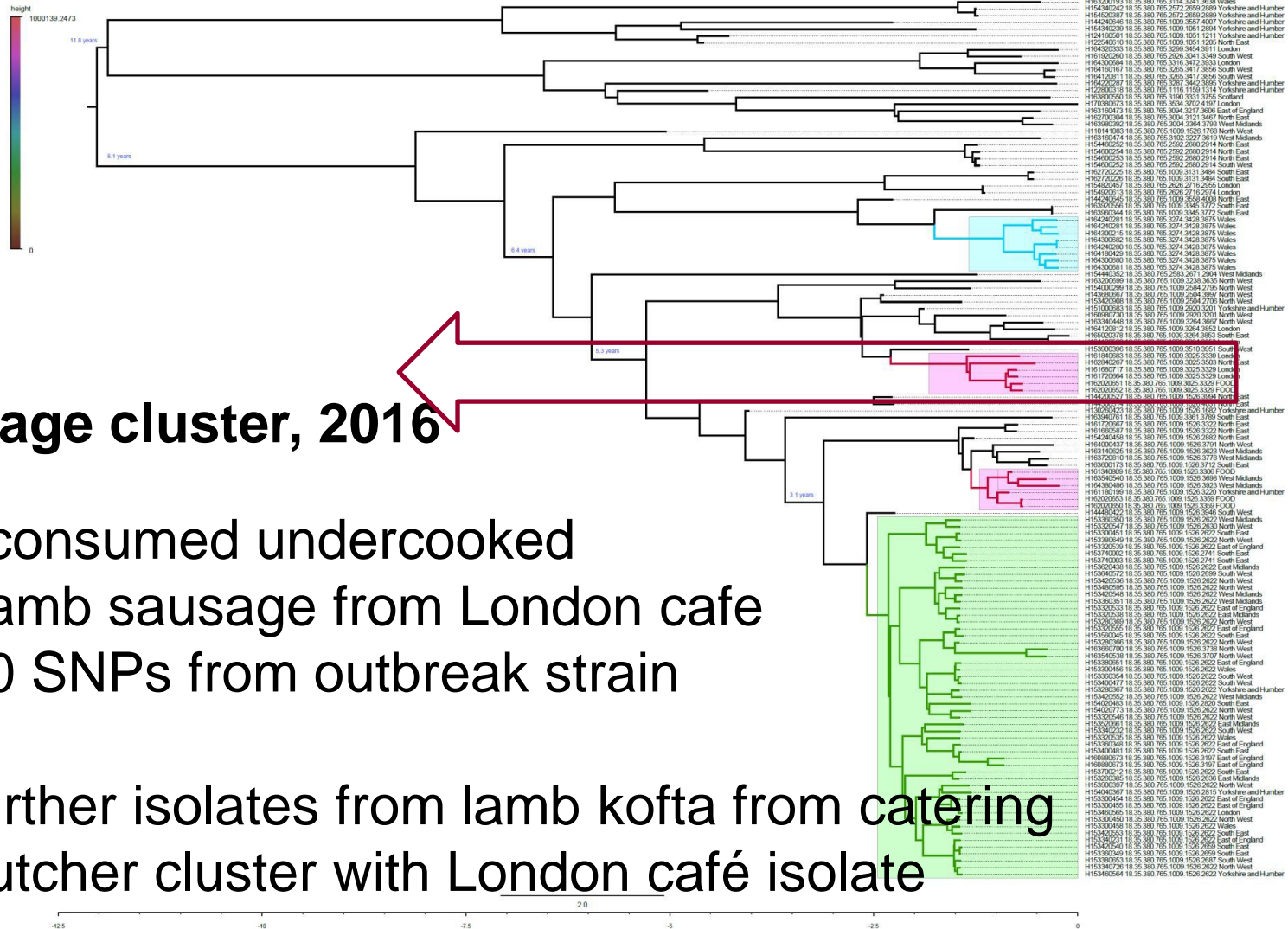
1. First case, cattle slaughter man, NW
2. Northern cluster: horse owner & nursery child NW, school child, NE
3. Case attended off road running event NW
4. Lake district cluster - child plays on farm next to knacker's yard, farm teacher with sheep, child walks in fields behind school
5. 3 cases school children in NW and NE all live near fields



Minced Lamb cluster 2016

- Minced lamb from national retailer sent routine testing
- Isolate from case that consumed the same batch of minced lamb
- Isolate from new lamb kofta product sent for shelf life testing from catering butcher

All cluster within 5 SNPs of outbreak isolates



Lamb sausage cluster, 2016

- Case consumed undercooked Lamb sausage from London café
10 SNPs from outbreak strain
- Two further isolates from lamb kofta from catering butcher cluster with London café isolate



O157 OB summary

- Strain initially detected during the prepacked salad outbreak August - October 2015.
- No new cases in this cluster until March 2016; marked difference in exposures and case demographics; minced lamb or live ovine exposures
- Four independent isolates from minced lamb products in the 10-SNP cluster;
- Increased and continuous activity of this strain to date; although more variation than seen previously, new cases within 5 - 50 SNPs of the original outbreak cluster continue to occur weekly.
- Origin of the prepacked salad outbreak likely to be ovine
- Questions remain around this strain amongst sheep in UK; how widespread is it?



Multicountry European Outbreak of listeriosis



Listeriosis is rare but severe disease with high fatality rate and causes the highest number of deaths due to a foodborne pathogen in Europe

L. monocytogenes isolates from 5 European countries have same WGS profile Nov 2017

Environmental isolate from French frozen food producer from surface used to prepare frozen vegetables including sweetcorn – matches by WGS Nov 2017

23 cases with food consumption data:

9 reported consumption of sweetcorn and 1 reported possible consumption

6 reported consumption of frozen sweetcorn

2 reported frozen vegetables, one non frozen both with sweetcorn

Sweetcorn consumption not part of food questionnaire for all MS — UK cases re-interviewed 3/4 sweetcorn consumption



On going multicountry European outbreak of listeriosis identified by WGS June 2018

Country	Number of cases (No deaths)				Total number of cases	Total number of deaths
	2015	2016	2017	2018		
Austria	0	2 (1)	0	0	2	1
Denmark	0	0	2	2 (1)	4	1
Finland	0	4	10 (2)	10	23	2
Sweden	0	3 (1)	3	1	7	2
The United Kingdom	1	2	2	6	11	2
Total	1	11 (2)	17 (3)	19 (1)	47	8



Jan and Feb 2018



L. monocytogenes detected in batches of frozen sweetcorn from Finnish trader (50-140cfu/g) with same WGS as OB strain

Sweetcorn produced in Hungary and packed in Poland

Recent Finnish case reported eating frozen mixed veg containing sweetcorn – same brand as above

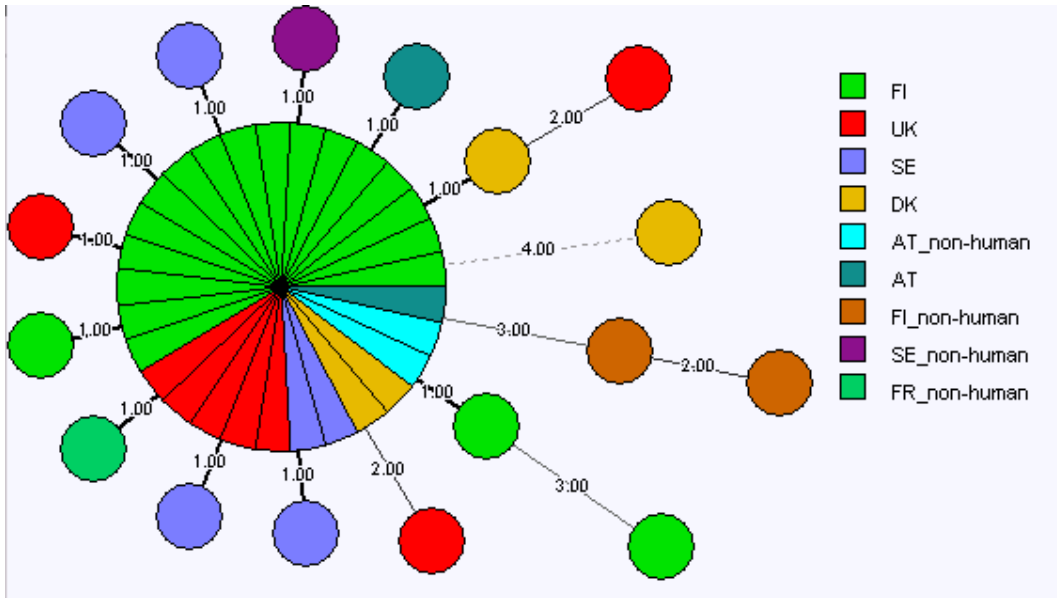
Swedish case reports eating frozen sweetcorn - brand unknown

Isolate with same WGS detected unacceptable levels in sweetcorn from home of a consumer in Sweden – traced back shows packed in Poland and produced in Hungary

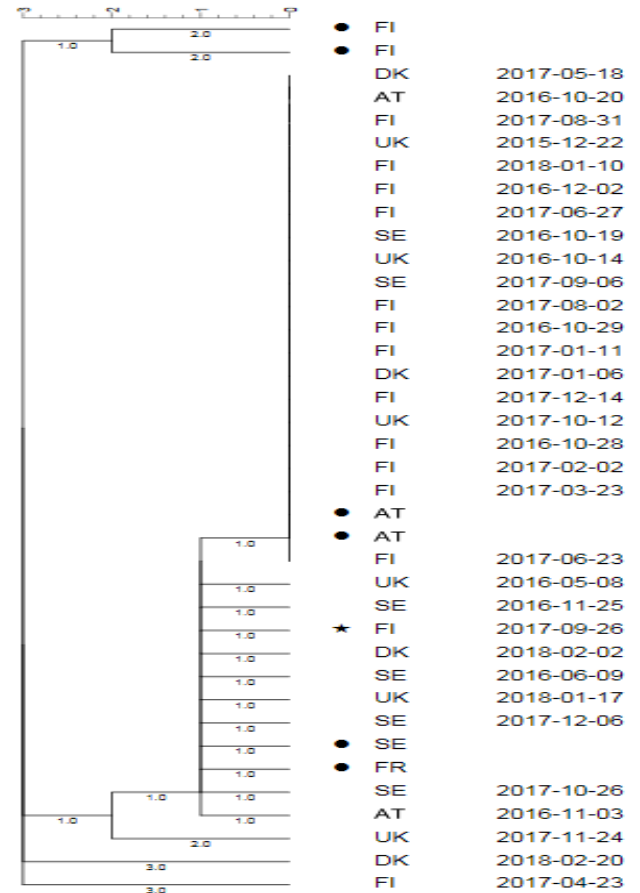
Austria reports isolates from 2 different types of frozen mixed vegetables including sweetcorn that originates same company in Hungary



Clonal complex 6

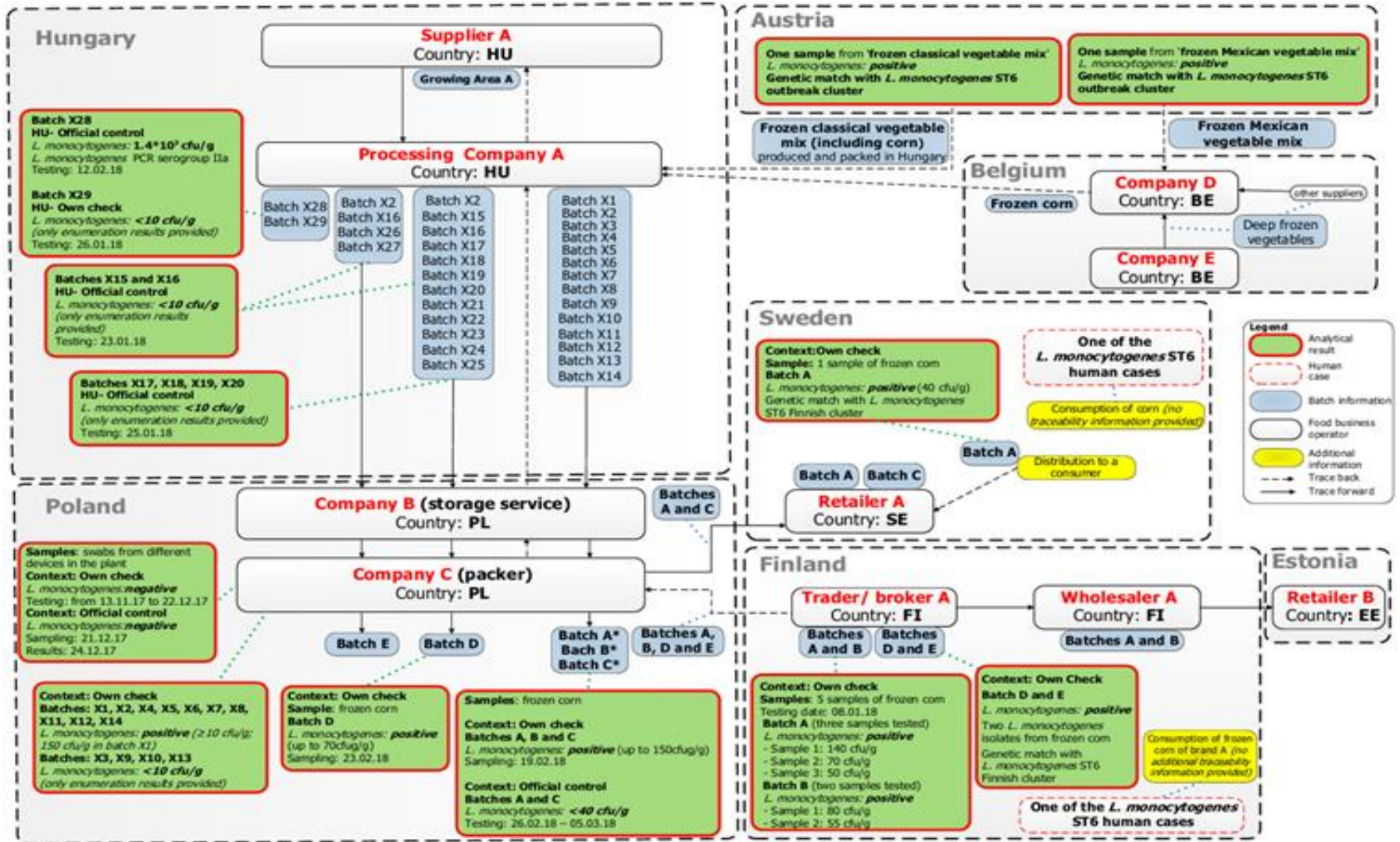


WGS_AlleleIds (Core loci)





Frozen sweetcorn investigations





Food investigations UK (11 cases)

Company supplying sweetcorn is large European-wide company supplying fresh and frozen vegetables + other products to major retailers, food service companies and industry across Europe

10 production sites across Europe including 2 in UK that supply a range of major supermarkets and food service providers many of which have been mentioned or have links with one or more cases in the UK

Frozen Sweetcorn from implicated Company in Hungary is supplied to the 2 production sites in the UK and thence to various supermarket and food service suppliers and is known to be supplied to hospital where one of UK cases was inpatient

Two recent cases have major supermarket branded frozen sweetcorn originating from Hungary in their home freezers. Supermarket known to be supplied by UK production sites – sampling and testing underway



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Summary of ongoing listeriosis outbreak



First multicounty European outbreak of listeriosis identified by WGS

Provides unequivocal evidence that isolates are related and come from a common source and have been present in food chain for 2-3 years

Isolates from frozen sweetcorn originating in Hungary have same WGS profile isolates from cases

Need to implement effective control measures to prevent more cases – recalling contaminated product

Improve communication on preventing consumption of uncooked frozen sweetcorn



WGS summary

- WGS is being used in real time for monitoring *Salmonella*, STEC, *Shigella*, *Campylobacter* and *Listeria monocytogenes*
- Highest degree of resolution for typing plus phylogenetic information – unequivocal evidence that strains are related and from a common source
- Improved surveillance and outbreak detection and investigation:
 - Real time monitoring of clusters, of virulence and AMR of all strains
 - Detecting more outbreaks – smaller OBs, geographically spread, over longer time frame, international outbreaks
 - Accurate and robust outbreak definition – finds cases and rules out unrelated cases from outbreak – refines OB investigation
 - Increased case ascertainment and indication of location/source of infection

WGS is improving public health interventions for controlling foodborne pathogen illness and the monitoring of their effectiveness



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Acknowledgements

All staff in GBRU and all those in Gastrointestinal Infections
Department

PHE Food and Water Laboratories and NHS hospital clinical
laboratories and PHE regional labs

PHE Genomic Services Unit and Bioinformatics Unit

PHE Health Protection Teams, Field Epidemiology Services

Food Standards Agency, Animal and Plant Health Agency

Local Authorities and Environmental Health Officers

ECDC, EFSA and all EU member states involved in outbreak
investigations