



Public Health
England

WGS of isolates from UK retail chicken and phylogeny in relation to time and processor

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Public Health
England

This talk

- Background
- Methods
- Results and discussion
- Further work



Human cases for England

Year	England	
	N	Rate*
2006	43806	86.0
2007	48622	94.6
2008	47096	90.9
2009	54438	104.3
2010	59200	112.5
2011	60616	114.1
2012	61255	114.5
2013	55906	103.8
2014	58782	108.2



Enumeration of *Campylobacter* on UK retail chicken projects

Projects funded my national competent authority (FSA) –
“Microbiological survey of *Campylobacter* contamination in fresh whole UK produced chilled chickens at retail sale”

- FS241044 – February 2014 to March 2015
- FS102121 – July 2015 to July 2018 & August 2018-July 2020
- Enumeration of campylobacters for up to 4000 fresh whole chickens per year
- Retailers sampled in proportion to market share initially, then equally; final two years focus on non-major retail stores



Level of contamination found in retail chicken in project

Year of sampling	cfu of <i>Campylobacter</i> spp. per gram of chicken skin sample							
	<10		10-99		100-1,000		>1,000	
	%		%		%		%	
2014-2015	26.7		22.5		31.4		19.5	
2015-2016	40.1		23.5		25.8		10.6	
2016-2017	46.1		27.7		20.4		5.9	
2017-2018	41.8		25.6		23.6		9.0	



- ~ every 10th of positive attempted (over selecting isolates from organic and free-range chickens) for WGS
- 618 submitted for WGS
- Sequencing libraries preparation with Nextera fragmentation and tagging; assembled with the SPAdes genome assembler. Contigs uploaded and alleles assigned. Data were compared at 7 multi-locus sequence typing (MLST) loci using the BIGSdb software.
- Single nucleotide polymorphism (SNP) profiles were generated and a core SNP alignment for each cluster was generated using SnapperDB and recombination removed using Gubbins. Maximum-likelihood phylogeny analyses were generated for each cluster using RAxML to identify closely related isolates and to confirm the 5-SNP clustering as grouping within the same clade.



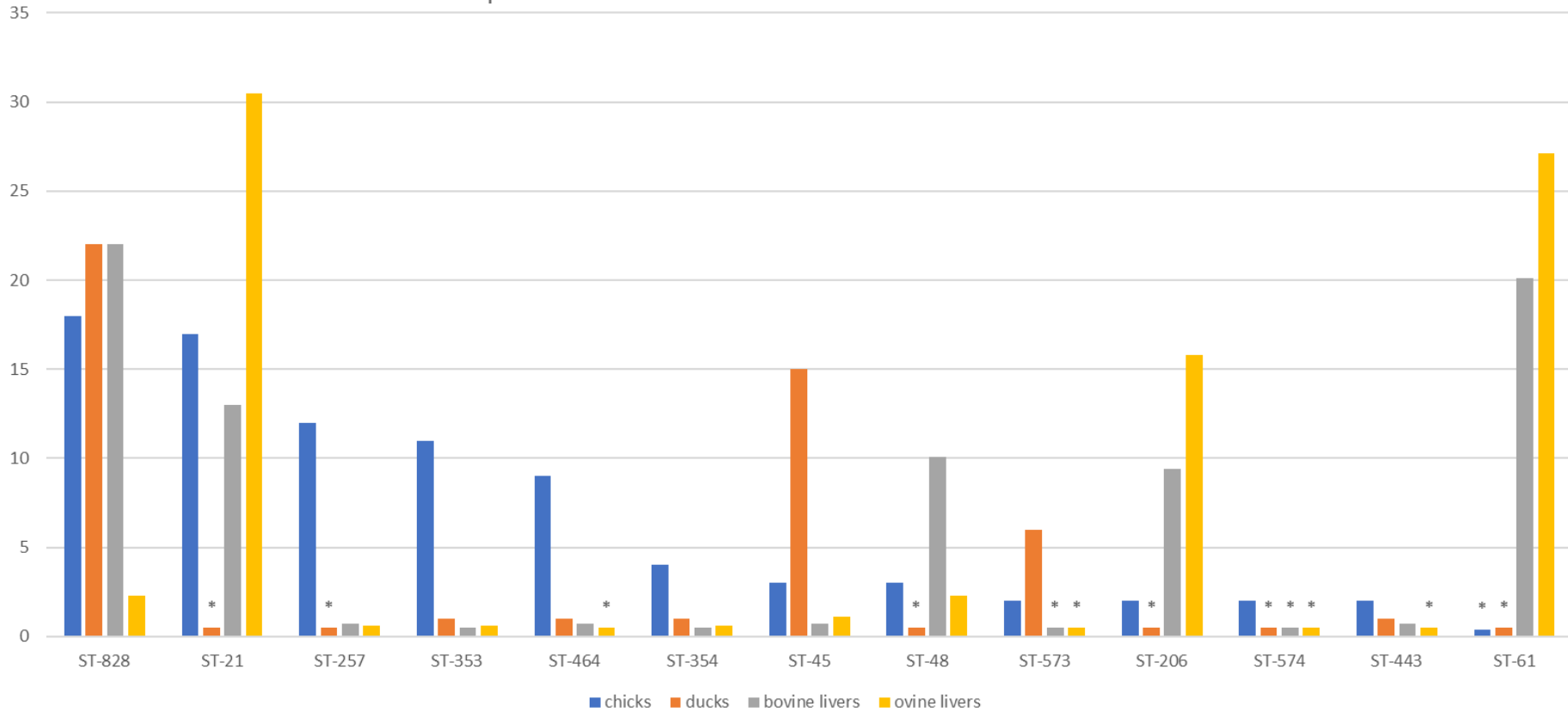
Results

- 83.2 % *C. jejuni* , 13.9 % of *C. coli* (3.6% both species – ie mixed pick)
- The proportion of samples *C. coli* was isolated from was significantly higher in summer compared to spring or winter months
- *C. coli* was detected more frequently in chicken reared as free-range/organic than in conventionally housed chicken
- WGS-based ST assigned to 613 isolates
- 135 different STs - majority assigned to 24 clonal complexes (CCs) but 63 isolates were not assigned to a CC
- Dominant CCs were ST-828, 21, 257, 353, 464, 354, 45, 48, 573, 206, 574 and 443. The most common STs were ST-828, ST-2254, ST-5136 and ST-50.



Abundance of Campylobacter CCs in chicken sample compared to other raw retail meat

Comparison of % of isolates in CCs in UK retail food sources





Results – types in retail chicken isolates

- CC21 was diverse with ST50 isolates most prevalent originating from at least 11 processing plants distributed throughout UK and were detected in all the years from 2015 to 2018.
- CC257 less diverse and dominated by ST2254 isolates detected in the years 2015 to 2018 and associated with 11 processing plants located throughout the UK.
- Isolates belonging to CC353 detected in samples from at least 9 processing plants and ST1036 and ST6461 accounted for just over half of CC353.
- CC464 was dominated by ST5136 isolates detected between 2015 and 2018, from 14 processing plants that were distributed throughout UK (except for NI)



Results – chicken SNP clusters

- In particular, there were highly similar isolates with average SNP-distances of 2, 6, 9 and 10 within ST44, ST6461, ST1036 and ST2254, respectively. ST50 had two separate clusters.
- CC21 ST44 isolates recovered from 2015 to 2018 were associated with five different processing plants; six isolates belonged to one 0-SNP cluster occurring in early winter 2018 and was associated with two plants located in the same region.
- One 0-SNP cluster of five ST50 isolates originated from the same plant and were recovered over five months from 2015 to 2016. Another 0-SNP cluster of ten ST50 isolates were recovered over three years and originated from four plants – three were closely located but one was distant.



Results

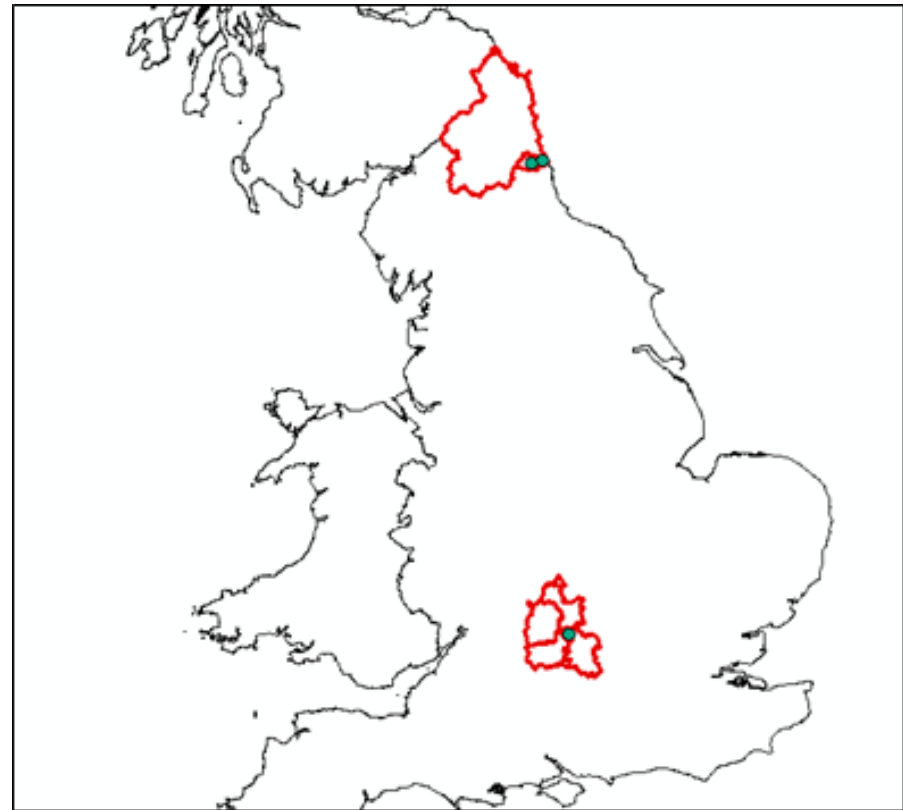
- In one CC257 ST2254 0-SNP cluster with three isolates detected over 3 months in early 2016 these were associated with the same plant - a further two isolates one SNP apart detected a month earlier were also from this plant.
- ST6461 isolates recovered in 2017 to 2018 were associated with at least six plants; there were two 0-SNP clusters and each was associated with at least two plants – and in one of these clusters the plants were located in different regions. The majority of ST1036 isolates were associated with the same plant but four isolates in one 0-SNP cluster detected over two years was associated with three distant plants. One ST353 0-SNP cluster was associated with two plants from the same region and recovered in the same year.



Isolates from human cases ...

How did they relate to chicken isolates?

- North East Site and
- Oxfordshire Site
- 2015 to 2018



265

Poultry associated campylobacteriosis in England stable for fifteen years: lamb's liver- an important cryptic source?

Prof. Noel McCarthy^{1,2,5}, Dr Melissa Janssen van Rensburg^{2,4}, Dr Alison Cody², Dr Craig Swift³, Dr Anais Painset³, Dr Tim Dallman³, Ms Clare Humphreys³, Dr Petra Manley³, Ms Amy Douglas³, Dr Alison Waldram³, Dr Frieda Jorgensen³, Prof Martin Maiden^{2,5}

¹University of Warwick, Coventry, United Kingdom, ²University of Oxford, Oxford, UK, ³Public Health England, UK, ⁴Imperial College, London, UK, ⁵NIHR HPRU in Gastrointestinal Infections, UK

Aim

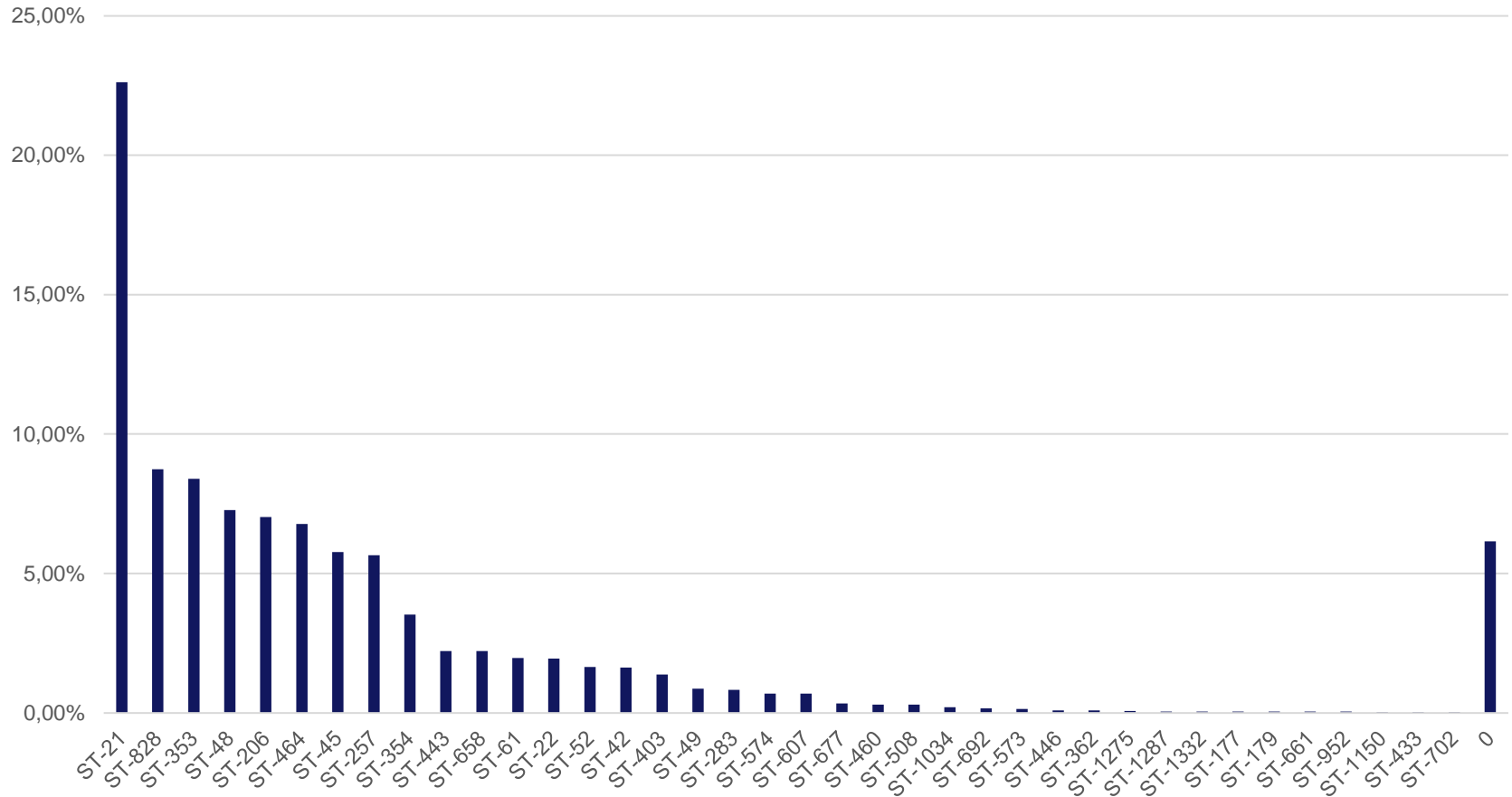
Identification of the sources of Campylobacter allows direction of control efforts and measurement of their impact, if any.

Methods

We conducted a systematic review of all published MLST genetic attribution studies. We then performed population genetic attribution in an English sentinel population followed for 15 years and comprising 9,010 cases. We used a large reference population of 9,916 isolates from putative sources, and adjusted for bias identified by self-attribution validation studies. We separately estimated sources of human infection based on extrapolation from sentinel types, sequence types restricted to humans and a subset of host reservoirs. We compared exposures among cases infected with sentinel types typical of different sources.



Proportion of human isolates in CCs (draft)





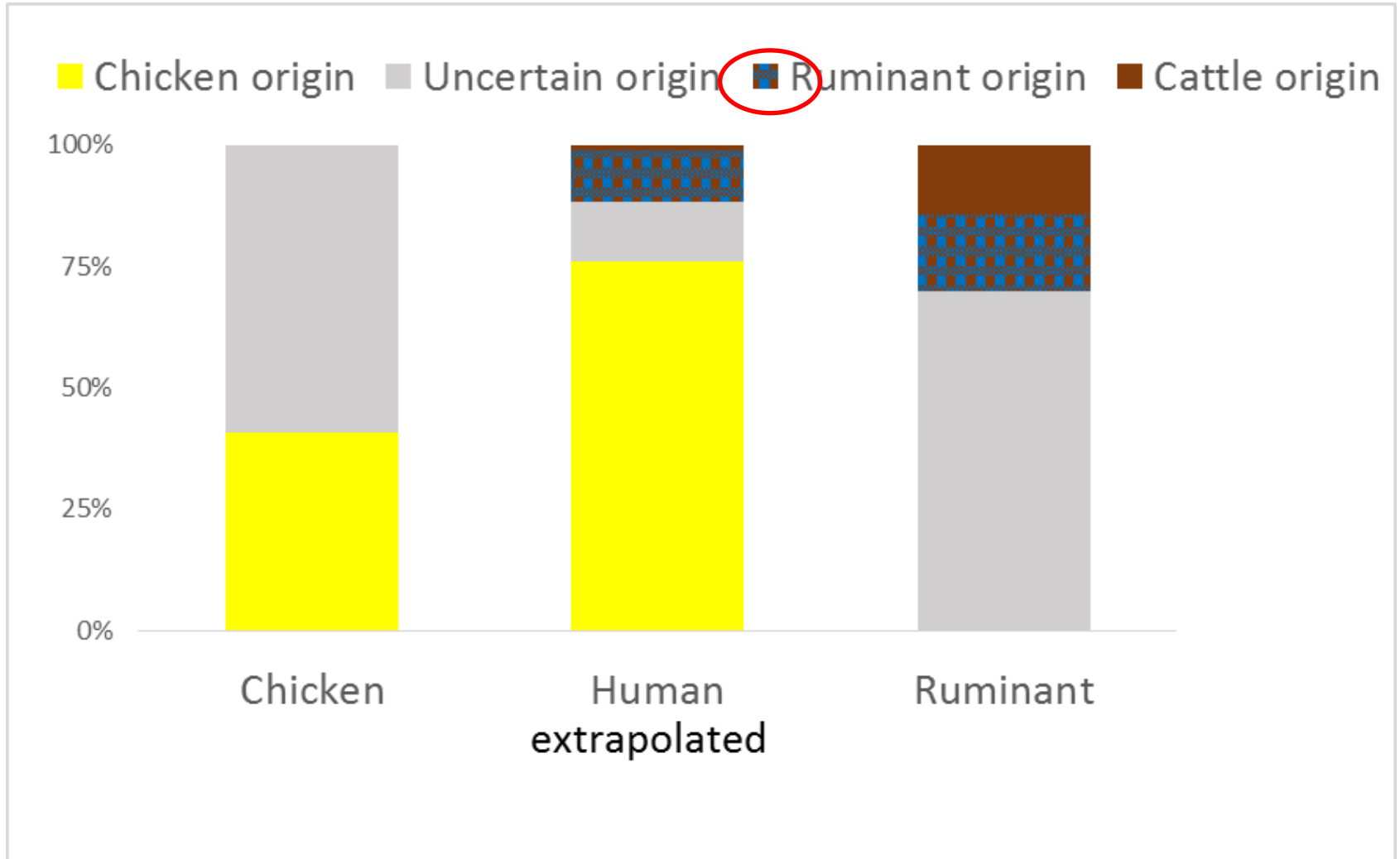
Preliminary attribution of campylobacter cases in England.

Analysis using an adjusted genetic attribution estimated 70-75% of English human cases were of poultry origin

- most other cases shared across cattle and sheep (the latter dominating over cattle-restricted types) – lamb's liver not insignificant.



Attribution analysis





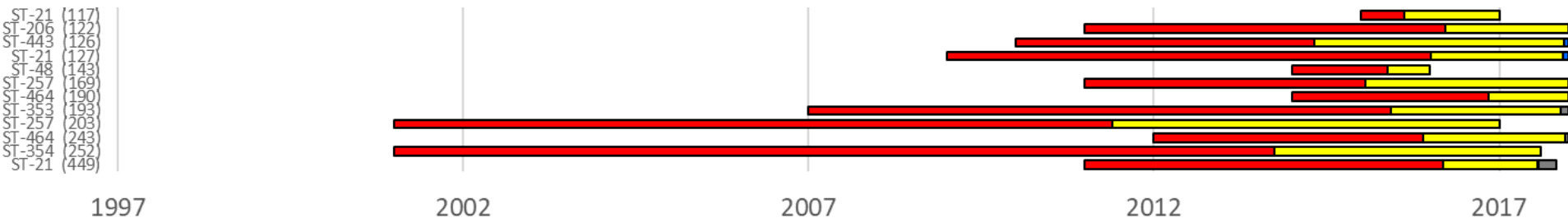
Discussion

- In comparison to the UK human cases, CCs ST206, ST21, ST403 and ST48 were less common in the sample from UK retail chicken.
- CCs ST573, ST257 and ST574 were more common in the retail chicken in comparison with the human cases.
- While 0-SNP chicken isolates clusters were often associated with the same plant and could occur over a short time frame, there were examples of 0-SNP isolates that did not associate with the same plant could be recovered over a long time scale.



Discussion

- Just over half of human cases related to ~ 17 % of the many (~1800) clusters detected
- Chicken was the predominant source in ~ 90% of SLC* and present in the five largest human clusters (ST50, ST354, two ST5136 and ST2030) – there was evidence of some clones disappearing (e.g. ST2030) at the same time in chicken and humans



McCarthy N.D. S. Hedges, C. Swift, A. Painset, T. Dallman, M.C.J. Maiden, A.J. Cody.
Use of core genome MLST analysis to infer the source of clustered clinical isolates. Poster
CHRO 2019.



Conclusion

- Lots of diversity in UK chicken isolates
- 0-SNP clusters are more likely than not to be associated with plants in the same area but not exclusively so
- Some 0-SNP clusters appeared short-lived but there was also evidence of persistent 0-SNP clones.
- Matches to the biggest human clusters were detected

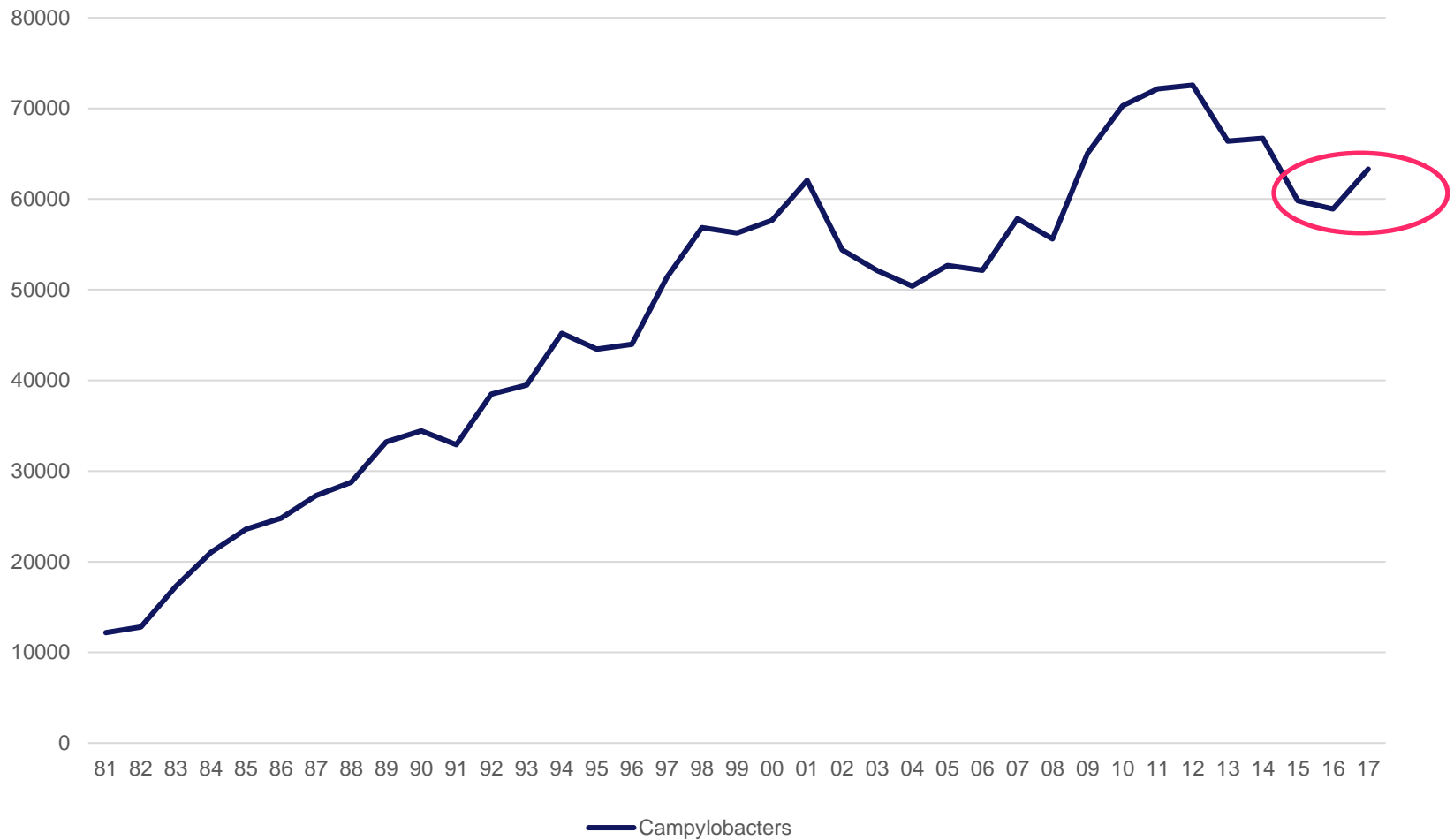


Further work

- Complete statistical analysis of extent to which chicken isolates belong to major human clusters detected
- Risk modelling using quantitative data associated with sequence type
- Write-up.



Campylobacter the most common bacterial cause of diarrhoea





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2014	58782	108.2
2015	51912	95.6
2016	48884	88.4
2017	53068	95.4
2018*	57674	103.7

Preliminary data from 2019 suggest a similar or slightly lower number of cases reported (final numbers delayed due to COVID19)

In 2020, so far, reported number of cases are lower compared to same time-frame last year – but this is probably due to COVID19...

*preliminary data



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Acknowledgements

University of Oxford: Alison Cody

Warwick University: Noel McCarthy and Ella Rodwell

AFBI Northern Ireland: Bob Madden, Nicolae, Pam and Jane

PHE: Craig Swift, Anais Painset and FW&E laboratories

Food Standards Agency for funding



Thank you for listening – Any questions?

Control of *Campylobacter* infections

- Reduce prevalence in chicken broilers
- Prevention of contamination of chicken meat at slaughter
- Prevention of cross-contamination in domestic and commercial kitchens from raw poultry meat and offal
- Adequate cooking of poultry and offal
- Adequate water treatment
- Pasteurization of milk
- Control recreational/occupational exposure

