i-CaMPS3
impact of interventions -
Campylobacter MLST Project in Scotland

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“Employing Source Attribution and Molecular Epidemiology to measure the impact of interventions on human campylobacteriosis in Scotland”

Final Report
April 2012 – March 2015
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Lay Summary

The Background
This study was commissioned by Food Standards Scotland (FSS) to improve our understanding of the most important causes of Campylobacter infection in humans in the Scottish population and to look at the potential impacts of interventions on campylobacteriosis. Campylobacter is the number one cause of bacterial infectious intestinal disease (IID) in the UK and cases of Campylobacter infection in Scotland remain at an all-time high. Campylobacteriosis in the Grampian Region has been studied in depth since 2005. Over this timeframe it has been representative of cases in Scotland as a whole although in 2015 incidence has declined from one of the higher rates to one of the lowest in Scotland. Previous studies funded by the Food Standards Agency (FSA) (CaMPS, i-CaMPS) have indicated that retail chicken is the largest contributing source of human infection caused by Campylobacter. Although a smaller but important proportion can be attributed to cattle and sheep.

The Study
Over the three years leading up to March 2015, Campylobacter isolates have been collected from clinical cases (2,393 isolates) and from host reservoirs such as chicken, turkey, cattle, sheep, pig and wild birds in Grampian (2,429 isolates) using the same growth/culture medium. Isolates were characterised to identify strain types and molecular attribution models were used to attribute strains to specific host reservoirs. A questionnaire about symptoms and exposures was issued to all cases in order to better understand routes of infection, seasonal trends and the risk factors for human campylobacteriosis in Scotland.

The Findings
Campylobacter was isolated from all sources using the same methods as previous studies with retail chicken having the highest prevalence (82%). All sources continued to show very high levels of strain diversity indicating that new strain types continue to emerge. The genetic similarity between isolates from clinical and chicken sources was higher in comparison to all other sources. As an example of this similarity between clinical and chicken strains, strain ST5136 was first observed in retail chicken and in clinical isolates in 2011. By this current study it had become the 5th most abundant strain from clinical cases, the 3rd most abundant strain in retail chicken and also found in retail turkey, but was still undetected in any other source.

Source-attribution modelling in the current study using chicken, cattle, sheep, pig and wild birds indicates that chicken is the largest source for human infection at 55% to 75%, followed by 10% to 22% for sheep, 10% for cattle, 0% to 8% for pigs and 4% to 8% for wild birds.

Turkey isolates are genetically quite similar to chicken isolates and the attribution calculated with the inclusion of turkey reduced the proportional attribution to chicken. This study found that retail turkey meat was less contaminated than chicken and there was no spike in incidence at Christmas time. The Agriculture and Horticulture Development Board (AHDB) market intelligence data based on DEFRA, HMRC and ONS data suggests that chicken meat is more popular than turkey with per capita consumption of 30kg/individual. On the contrary turkey accounts for 3kg/capita consumption and the production has halved since 2000.
Hence, although turkey isolates are similar to chicken isolates, it is likely that the proportion of cases attributable to turkey is small.

Cases reporting contact with animals (8.3%) prior to their illness were more likely to have infection from a non-chicken attributable strain. Similarly, cases with a private water supply were less likely to be infected with a chicken attributable strain. Handling raw chicken gave a non-significant increase in the likelihood of infection with a chicken attributable strain. 19% of cases reported being abroad prior to their illness. 9% of cases were hospitalised following their *Campylobacter* infection.

The typical increases in the numbers of human cases seen in the late spring/summer and again in the autumn, in contrast to the lower incidence over the winter period were not due to changes in the proportions of cases attributable to the different sources. The cause of these seasonal variations is still enigmatic.

**The Conclusions**

The balance of evidence implicates retail chicken as the largest source of human infection followed by cattle, sheep, pigs and wild birds.
Glossary

**Allele**: An allele is a variant form of a gene with each allocated a unique number.

**Attribution**: Inference of the source of human *Campylobacter* infection using strain type.

**cfu**: Colony forming units. Typically a measure of the number of live cells in a sample.

**Clonal complex (CC)**: A group of sequence types (STs) whose members are linked to at least one other member by being identical for six of the seven MLST genes.

**DEFRA**: Department for Environment, Food and Rural Affairs

**HMRC**: Her Majesty's Revenue and Customs

**Host association**: The concept of a host species having a characteristic set of *Campylobacter* strains.

**Host reservoir**: Domesticated and wild animal and bird species, in which *Campylobacter* is maintained as a source of human infection.

**IID**: Infectious Intestinal Disease

**Isolate**: A *Campylobacter* culture isolated from a specimen by microbiological methods.

**MLST**: Multi Locus Sequence Typing.

**ONS**: Office for National Statistics

**Prevalence**: number of isolates belonging to the same species within a population. It is calculated as, total number of strains belonging to a specific species from a specific host ÷ total number of specimens collected from a specific host

**Sequence type (ST)**: For each housekeeping gene, the different sequences present within a bacterial species are assigned as distinct alleles and, for each isolate the alleles at each of the seven loci (location) of the housekeeping genes define the sequence type (ST).

Example:

<table>
<thead>
<tr>
<th>ST</th>
<th>aspA</th>
<th>glnA</th>
<th>gltA</th>
<th>glyA</th>
<th>pgm</th>
<th>tkt</th>
<th>uncA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>1</td>
<td>54</td>
<td>3</td>
<td>4</td>
<td>1</td>
<td>5</td>
</tr>
</tbody>
</table>

**Specimen**: A swab containing *Campylobacter* growth from clinical, food or veterinary sources, a sample of animal or bird faeces, or a portion of retail food.

**Strain**: A collection of genotypically indistinguishable isolates. Usually of a common ST.

**Type / typing**: Often genotyping or phenotyping. The process of characterising an isolate at the level of its DNA or at the level of its expressed biological characters (biochemical, physiological, antigenic etc.)
1. Introduction

1.1. Background

_Campylobacter: A foodborne pathogen_

_Campylobacter_ is the cause of almost half of all bacterial infectious intestinal disease (IID) cases with _Campylobacter jejuni_ causing around 90% of campylobacteriosis cases and the closely-related _Campylobacter coli_ causing almost all the rest (Acheson, Allos 2001, Tam, O’Brien et al. 2003). _Campylobacter_ infection was first implicated in causing human enteritis in the late 1970s (Skirrow 1977) and has since become recognised as the commonest known cause of bacterial IID worldwide. According to World Health Organisation (WHO) estimates, _Campylobacter_-related illness including post-_Campylobacter_ coeliac disease affects around 1% of the worldwide population (Green, Cellier 2007). _Campylobacter_ has also been isolated from 10% of presenting or relapsed cases of inflammatory bowel disease (IBD) (Navarro-Llavat, Domenech et al. 2009) and recent cohort studies have demonstrated a higher risk of IBD following acute _Campylobacter_ infection (Man 2011, Rodríguez, Ruigómez et al. 2006, Gradel, Nielsen et al. 2009). Human campylobacteriosis caused by _Campylobacter_ spp. therefore remains a global public health problem.

_Campylobacter_ is a Gram-negative pathogen that lives predominantly as a commensal in the gastrointestinal tracts of a wide range of animals and birds, including farmed and companion animals (Sahin, Kassem et al. 2015). It is a zoonosis and causes a range of symptoms in humans including self-limiting bouts of diarrhoea, abdominal cramps, fever, headache, nausea and vomiting; rarer conditions include arthritis and Guillain-Barré Syndrome (GBS) (Strachan, Forbes 2010). Approximately one of every 1000 reported cases in the UK leads to GBS: a serious condition of reversible or permanent loss of limb motor function that is the commonest cause of acute flaccid paralysis (Tam, Rodrigues et al. 2006). GBS is an illness that affects about 1500 people every year in the UK (Kenny, Tidy 2013) and is associated with serious longer-term illnesses due to extensive axonal injury, a greater likelihood of the need for mechanical ventilation, and a very high risk of irreversible neurological damage (Allos 2001). _Campylobacter_ infection is also associated with the non-paralytic version of GBS, Miller-Fisher syndrome, and with reactive arthritis. Other serious complications of _Campylobacter_ infections occur due to direct spread from the gastrointestinal tract and can include cholecystitis, pancreatitis, peritonitis, and massive gastrointestinal haemorrhage (Allos 2001). Although rare, extra-intestinal manifestations include meningitis, endocarditis, septic arthritis, osteomyelitis, and neonatal sepsis (Allos 2001).

There are 17 million cases and 1 million GP consultations due to IID every year in the UK (Tam, Rodrigues et al. 2012). IID incidence in the community has increased since the 1990s whilst GP consultations have halved. _Campylobacter_ is the major bacterial agent implicated in IID causing an estimated 700,000 cases and 80,000 GP consultations annually in the UK (Tam, Rodrigues et al. 2012, O’Brien 2012). In the year 2000, _Campylobacter_ caused 86 deaths and the cost to the UK economy was around £113 million (Advisory Committee: Food Safety 2005). Today, _Campylobacter_ causes more than 200 deaths per year with as many
as 80,000 consumers stricken by life-altering illnesses (Stones 2014, O’Brien 2012) and the costs to the UK economy are around £1bn each year (Ketley 2013). *Campylobacter* still remains a high priority in the UK, as highlighted by the scientific evidence and information strategy 2015-20 delivery plan by the Food Standards Agency (FSA 2016).

A 46% increase of *Campylobacter* confirmed cases has been observed in the UK between 2004 and 2012 (FSA 2013). During the same period, an estimated 39% rise was noted in England and Wales (Public Health England), a 62% rise in Northern Ireland (Public Health Agency) and a 43% rise in *Campylobacter* cases in Scotland (Health Protection Scotland). During 2015, 6,260 laboratory reports of *Campylobacter* were reported by Health Protection Scotland (HPS); a decrease of 5.66% compared to 2014 (n=6,636). For the last five years the number of reported Scottish cases has hovered at just over 6,000 per year, which followed a sustained period of increasing incidence (Figure 1). In 2014, the reported incidences across Scottish Health Boards was broadly typical of previous years (Figure 2). In Scotland the overall rate of *Campylobacter* infection was 117.1 per 100,000 in 2015 compared to 124.6 per 100,000 in 2014 (Figure 2, 3). Among the mainland NHS boards, the rates of *Campylobacter* ranged from 70.2 per 100,000 to 147.7 per 100,000 which is less than the 76.0 per 100,000 to 162.8 per 100,000 reported in 2014. The decline in *Campylobacter* throughout 2015 was observed in all mainland health boards apart from NHS Lothian and NHS Borders (mainland boards) (Figure 2). It was observed that the rates of *Campylobacter* infection were higher in children under five and among those aged 50 years and older (Smith-Palmer, Brownlie 2016). The greatest percentage increase in 2014 was among those aged 0-4 years, an increase of 25% from 284 reported cases in 2013 to 355 in 2014 (Smith-Palmer, Brownlie 2015). Although *Campylobacter* is the most common bacterial cause of IID, most cases are sporadic (Tam, Rodrigues et al. 2012). General outbreaks of *Campylobacter* continued their usual pattern of being rare (as reported to ObSurv, the surveillance system for general outbreaks of infectious intestinal disease in Scotland) with 34 recorded outbreaks from 1996 to 2014: none recorded in 2015 and one in 2014 (Smith-Palmer, Brownlie 2015).

The trends in overall incidence of Scottish campylobacteriosis continue to be broadly mirrored by those in Grampian, as they have done over the past 25 years (Figure 3) therefore Grampian will be the focus of the study due to its adequacy to represent Scotland as a whole. Although, it is worth noting that a decrease in the level of incidences in Grampian has been observed in 2015 compared to Scotland as a whole (Figure 3).

Within Grampian (Figure 4), age stratification generally showed increase in incidence across most age groups compared to previous years. There continues to be six age groups (5-9, 10-14, 15-19, 30-34, 35-39, 40-44) which have lower incidences than the overall average (Figure 4). The highest incidence of campylobacteriosis is seen in young children (0-4), young adults (20-24) and the elderly (60-64) (Figure 4).

**Food Standards Scotland has designated *Campylobacter* as of Strategic Importance**

Most foodborne illnesses are caused by the consumption of food which has become contaminated with pathogenic bacteria. Hence, protecting consumers in Scotland
from the risks of foodborne illness is a key priority for FSS. Retail chicken is the main source of human campylobacteriosis in Scotland (Miller, Dunn et al. 2004, Sheppard, Dallas et al. 2009) and in the developed world (Crushell, Harty et al. 2004, Kaakouch, Castano-Rodriguez et al. 2015). A significant proportion of the remainder is attributable to ruminants (Strachan, Forbes 2010, EFSA 2010, Little, Gormley et al. 2010, Nelson 2010, Tustin, Laberge et al. 2011).

An ongoing study conducted by the Food Standards Agency (FSA) from Feb-2014 looked at the prevalence and levels of Campylobacter contamination in whole chilled chickens and their packaging. The survey tested more than 4,000 samples of whole chickens bought from UK retail outlets and smaller independent stores and butchers (FSA 2015b). The results showed that 73% of chickens tested positive for Campylobacter with 19% of chickens positive for Campylobacter within the highest level of contamination (>1000 cfu/g). Of the packaging tested 0.1% (n=5) was positive at the highest level of contamination and 7% of packaging tested positive for the presence of Campylobacter (FSA 2015b).

The UK and Scottish governments have a responsibility to promote health and maximise health care efficiencies, and therefore require the incidence of human Campylobacter infection to be substantially reduced. Food safety regulatory bodies and organisations in the food production sector are well-placed to identify and implement effective interventions. ACT (Acting on Campylobacter Together) aims to identify interventions that would reduce Campylobacter in chicken. These bodies include the British Poultry Council (BPC), the National Farmers' Union (NFU) the British Retail Consortium (BRC), all major supermarkets, the FSA, Food Standards Scotland (FSS) and DEFRA. Collectively, their aim is to identify and put in place interventions that will reduce Campylobacter through a Joint Action Plan. The key activities of the action plan relate to on-farm, transport, processing, retail, consumer and catering sector trials and interventions, as well as surveillance and monitoring.

Measures proposed by food safety regulatory bodies for mitigating Campylobacter include:

On farm: free Campylobacter testing service for farmers in order to incentivise producers to examine their biosecurity procedures. Rapid detection of Campylobacter using a real time robotic machine; more informed bio-security measures; feed additives to reduce adhesion of Campylobacter; stopping thinning; cameras to detect changes in chicken behaviour following colonisation.

At slaughterhouse: Rapid surface chilling; steam and ultrasound; secondary scalding.

At retail: Roast-in-bag packaging and don’t wash your raw chicken education campaign.

The impact of these on public health has yet to be determined.

Previous studies funded by FSS (formerly Food Standards Agency) on Campylobacter in Scotland include CaMPS (2005-2007), i-CaMPS-1(2010-2011) and i-CaMPS-2 (2011-2012). The current study (FSS00017) commissioned by FSS, improved our understanding of the most important causes of Campylobacter in humans in the Scottish population. The studies above identified that chicken, cattle and sheep sources were of greatest relevance. The lack of information on the prevalence and genotypes of Campylobacter in pigs and in retail turkey from the previous studies was identified as a gap, hence turkey and pig sampling was...
done in the current study in addition to chicken, cattle, sheep, and wild birds in order to elucidate their role in human campylobacteriosis.

Previous studies funded by the Food Standards Agency established a number of methodological criteria relevant for the current study:

- Grampian is representative of Scotland in terms of attributing the sources of human campylobacteriosis.
- Larger host datasets are more informative and robust for attribution than smaller datasets.
- The five variant molecular attribution models implemented gives broadly the same source attribution results, with Asymmetric Island showing a higher proportion of attribution to chicken. Accordingly the current study has focused on the STRUCTURE with alleles and Asymmetric Island models.
Figure 1. Annual incidence of campylobacteriosis based on cases reported in UK.

Red line indicates *Campylobacter* cases reported to the PHE (HPA) for England and Wales from 1989-2015 and includes patients with enteric and non-enteric infections and includes isolates from all body sites. (https://www.gov.uk/government/collections/campylobacter-guidance-data-and-analysis).

Blue line indicates laboratory isolates of *Campylobacter* reported to HPS from 1989-2015 (http://www.hps.scot.nhs.uk)
Figure 2. Incidence per 100,000 population of reports of *Campylobacter* infection 2015 (2014) based on reported cases.

Data from Health Protection Scotland (http://www.hps.scot.nhs.uk/).
Figure 3. Incidence of campylobacteriosis in Scotland and Grampian from 1990 to 2015 based on reported cases.

Data from Health Protection Scotland (http://www.hps.scot.nhs.uk/). Error bars are 95% CI calculated by bootstrapping.
Figure 4. Age structured incidence of campylobacteriosis in Grampian for 2005-07 and 2010-15 based on reported cases.

Data from Health Protection Scotland (http://www.hps.scot.nhs.uk/). Error bars are 95% CI calculated by bootstrapping.
1.2. Aims

Rationale: The contemporaneous sampling and collection of *Campylobacter* isolates from human, food and environmental sources, in combination with the molecular genotyping isolates and use of state of the art molecular attribution modelling has allowed an integrated and quantitated determination of the relative importance of the different sources of *Campylobacter* on the incidence of human disease. This study will characterise and perform source attribution of campylobacteriosis in Grampian from April 2012 – March 2015. This will be based on clinical isolates obtained from cases, poultry and farm animal isolates. The results will allow the FSS to appraise, on a population level basis, the efficiency of any interventions being brought in by industry in conjunction with the UKs ‘Acting on *Campylobacter* Together’ (ACT).

The aims of this study are to:

1. Clarify the sources of human campylobacteriosis in Grampian, Scotland during Apr 2012 – March 2015
2. Use next generation sequencing approach in order to type isolates belonging to specific host reservoirs.
3. Use source attribution models on isolates from appropriate sources typed by Multilocus Sequence Typing (MLST) to understand the role of host reservoirs in infection caused by *Campylobacter*.
4. Understand the specificity of strains belonging to host reservoirs and their dynamics.
5. Identify routes of infection, seasonal trends and the risk factors for human campylobacteriosis in Scotland.
6. Compare the findings of the current study with previous studies: CaMPS study of 2005-2007 (Forbes, Horne 2009), i-CaMPS 2010-present.
7. Provide baseline data against which the success of future ‘farm to fork’ interventions in the broiler chicken industry can be measured.
2. Materials and Methods

2.1. Objective 1: Collection of isolates

All available clinical isolates in Grampian for the period Apr 2012 – Mar 2015 (n=2,393) were received from Aberdeen Royal Infirmary diagnostic microbiology laboratory (Table 3). Contemporaneous *Campylobacter* isolates from the principal source hosts were also collected (Table 3). Retail chicken and turkey were sourced from shops around Aberdeen on a Tuesday, stored at 4 °C and processed on Wednesday. Cattle, sheep and pig faecal samples were collected by Food Standards Agency Scotland (FSAS) Operations staff at Portlethen, Turriff and Brechin abattoirs on a monthly basis. The samples were collected on Friday and Monday, stored in a refrigerator and couriered on Tuesday in cool boxes with cooling gel packs to the lab at the University of Aberdeen. The source of the originating animals was selected to be predominantly from North East Scotland and from a variety of farms.

The *Campylobacter* isolates from animal and food sources were cultured at the University of Aberdeen by microbiological culture from faecal or food specimens. Animal faecal specimens (25g) were homogenised in 225 ml of *Campylobacter* enrichment broth. Each food specimen was incubated at ambient temperature in enrichment broth for 1 hr with occasional agitation; the volumes used were 300ml for portions and 500 ml for whole carcasses. Further details of isolation and culture was carried out and has been previously published (Forbes, Horne 2009).

Wild bird faeces were collected by several volunteers, mostly as part of ongoing bird ringing studies from Grampian. Only excreted faeces was collected; never by invasive sampling as this is prohibited by the regulatory body (British Trust for Ornithology). Faeces was collected on charcoal swabs and couriered or posted to the University of Aberdeen. The swabs were streaked directly onto both modified charcoal-cefoperazone-deoxycholate (mCCDA) and blood agar plates and were also put into an enrichment medium in a universal tube (around 20 ml). Both plates and enrichment were incubated for 48 hours. After 2 days, plates were checked for visible colonies of *Campylobacter*. If plates were negative, enrichment was then plated to check for the presence of *Campylobacter* following incubation at 37ºC for 48 hrs under microaerobic conditions.

Enumeration of *Campylobacter* from faecal and food specimens was by direct plating, and further isolations made after extended growth in enrichment broth. For direct plating, 0.1 ml of neat and a 10-fold dilution for both faecal homogenates and food portion washes were plated onto mCCDA plates, and the plates incubated as below. For enrichment growth, the remaining volume of the faecal homogenate or food portion wash was incubated under microaerobic conditions at 37ºC for 2 days. Samples (0.1 ml) were removed, plated onto mCCDA plates, and the plates incubated as described. The presence or absence of *Campylobacter* colonies was determined visually and confirmed by visible agglutination with Microscreen *Campylobacter* latex confirmation assay (product code M46, Microgen Bioproducts). Colony growth from both isolation procedures was harvested, archived and prepared for DNA extraction as described in section 2.2.
2.2. Objective 2: Whole genome sequencing, Genomic DNA extraction and Strain typing

The preparation of whole genomic DNA which is suitable for Whole Genome Sequencing (WGS) utilised the Promega Wizard Genomic DNA Purification Kit (Catalogue #A1125).

All of the DNA samples went through a genomic library prep which is similar to the Illumina Truseq protocol developed by the Sanger Institute. The libraries were sequenced on Illumina HiSeq 2000 analysers on 100bp paired end runs. Typically, a single lane which has 90 to 96 multiplexed samples produced 40Gb data (in the form of 100bp reads). Assuming the data is distributed evenly between all the samples, each isolate will produce about 400Mb. The paired read files were de novo assembled using the Velvet assembler via an established pipeline at Sanger Institute (Figure 5).

**Bacterial Isolate Genome Sequence Database (BIGSdb):**

The sequences were imported into BIGSdb (Bacterial Isolate Genome Sequence Database (Jolley, Maiden 2010), a bioinformatic pipeline developed at University of Oxford. BIGSdb is software designed to store and analyse sequence data for bacterial isolates. Any number of sequences can be linked to isolate records - these can be small contigs assembled from dideoxy sequencing through to whole genomes (complete or multiple contigs generated from parallel sequencing technologies such as 454 or Illumina Solexa). All the functionality of mlstdbnet and agdbnet software has been incorporated into BIGSdb and this software will be used to eventually host all the databases on the PubMLST.org site. BIGSdb extends the principle of MLST to genomic data, where large numbers of loci can be defined, with alleles assigned by reference to sequence definition databases (which can also be set up with BIGSdb). Loci can be grouped into schemes so that types can be defined by combinations of allelic profiles, a concept analogous to MLST.

**Strain typing:**

Multilocus sequence typing (MLST) is a technique for characterising isolates of bacterial species using the sequences of internal fragments of (usually) seven house-keeping genes (Maiden, Bygraves et al. 1998). Most bacteria have sufficient variation in their house-keeping genes to provide many alleles per locus, allowing billions of distinct allelic profiles to be distinguished using seven house-keeping loci (Urwin, Maiden 2003). MLST and the related method of multilocus sequence analysis (MLSA) (Gevers, Cohan et al. 2005) are advantageous as they have a high resolving power. The great advantage of MLST is that sequence data are unambiguous and the allelic profiles of isolates can easily be compared to those in a large central database using the internet (Urwin, Maiden 2003). The isolates can be precisely characterised even if they cannot be cultured from clinical material (Urwin, Maiden 2003).

The whole genome sequences of the isolates were used to classify them to species level, and for *C. jejuni* and *C. coli* into strain types using 7-locus MLST (Jolley, Bliss et al. 2012). Allele numbers and sequence types (ST) were assigned using the public *Campylobacter* PubMLST database http://pubmlst.org/Campylobacter/. Multi-locus Sequence Typing was carried out on all isolates and this is summarised...
in Table 3. Not all presumptive isolates were confirmed to be *Campylobacter jejuni/coli* by MLST and this was due to the challenge in confirmation by visual inspection of colonies and false positive results by latex sero-agglutination.
Figure 5. Sanger Institute bioinformatics assembly pipeline for whole genome sequencing of *Campylobacter* spp gDNA.
2.3. Data archiving

Central to the functionality of this study, and its linkage to the previous studies, is the collation of all project data into a single Microsoft Access database (Figure 6). This includes the metadata associated with the original specimens, the resultant microbiology, the genomic metadata, the typing data and also the associated case questionnaire data. This relational database allows the linkage of disparate fields which enables subsequent analyses. The database is backed up regularly and images are archived prior to the addition of new data. All of the sequencing raw read files (fastq format) are uploaded to the publicly available database at the European Bioinformatics Institute (EBI).
Figure 6. Information and Fields held in iCaMPS Access database.
2.4. Patient questionnaires

Questionnaires have continued to be sent to all cases in Grampian by the Health Protection Team of Grampian Health Board. A total of 19 questions were asked out of which 11 were close-ended questions. A sample of the questionnaire can be found in the annexure.

The information collected by the questionnaire is as underlined below:

1. Basic information on who was ill: age, gender, first 5 digits of the post-code, employment status.

2. Basic information on illness: start date and end date of symptoms, type of symptoms experienced (diarrhoea, bloody stools, vomiting, abdominal pain or other symptoms), if the subject was admitted to the hospital and if anyone in the household were ill during that time.

3. Foreign travel: Information on if the subject had overnight stay abroad and if anyone else in that area experienced similar illness.

4. Exposure: Cases were asked if they had contact with animals, had a private water supply, if they or anyone else in the household had handled raw chicken, eaten chicken or chicken liver pate, if chicken was purchased and prepared at home and which supermarket it was bought from, in the five days before onset of illness.

Aberdeen Royal Infirmary laboratory provided information on patient age, gender, post-code and sample received date. The questionnaire information had the date of birth of the patient, age, gender, start and end date of the illness and date on which the form was completed. This information was used to link the questionnaire (case) with a specific isolate.

The information obtained from the paper questionnaires was input into an Access database and then processed in excel for performing empirical analyses.

2.5. Objective 3. Analyses

Task 01a. Molecular characterisation of source and clinical Campylobacter isolates: Host reservoir isolate datasets

In previous reports issued on projects commissioned by FSA, food and animal isolates from the 2005-06 Scottish study and the i-CaMPS 2010-12 period were used in the molecular attribution analyses (Table 1). This was because there were insufficient isolates to significantly change the resultant attribution scores and enable comparison of attribution results with the previous report. Strain prevalences change over time (Ogden, Dallas et al. 2009) however not incorporating contemporaneous data is not expected to alter findings significantly.

This report makes use of the i-CaMPS-3 host dataset which includes turkey and pig isolates as well as chicken, cattle, sheep and wild bird isolates from 2012-15 (Table 1). We expected Campylobacter prevalence in turkey to be high – comparable to chicken - but in fact it is about half the prevalence to that observed
in chickens (Table 3). This lower prevalence is of note in interpretation of the attribution studies.

In the current wild bird testing only 6/84 samples were positive for *Campylobacter jejuni/coli* and so isolates collected from 2005 - 2007 have been used in this study. Low prevalence has also been observed in other studies that looked at the prevalence of *Campylobacter* in wild birds of the mid-Atlantic region, USA and in Northern England (Keller, Shriver et al. 2011, Hughes, Bennett et al. 2009). The prevalence of *Campylobacter* in wild birds is linked to various ecological and phylogenetic factors, with great variations in carriage between different taxa and guilds (Waldenstrom, Broman et al. 2002).

*Campylobacter lanienae* was isolated frequently from pig faeces, and previous reports identified this host specificity with *C. lanienae* strains but not with cattle or broilers (SASAKI, FUJISAWA et al. 2003). Over the winter there was a steady decline in *Campylobacter* isolates that were retrieved from pigs therefore sampling was increased to twice per month to facilitate obtainment of >100 *Campylobacter* isolates. All isolates identified were either *C. coli* or *C. lanienae*.

**Task 01b. Molecular characterisation of source and clinical *Campylobacter* isolates: Descriptive analytical methods**

The following methods were employed to characterise the strain diversity from various sources (humans, chicken, turkey, cattle, sheep and wild birds) over the different time periods (2012-2015, 2005-2009 for wild birds) used in this report.

Graphical visualisation shows the distribution of strains over time and by source.

Fisher’s exact test allows comparison of the relative abundance of the most prevalent strains over time.

Rarefaction is used to characterise the isolates from sources represented the maximum hypothetical diversity. It is a data re-sampling technique that indicates whether diversity has reached a plateau or is still rising at the total sample size, i.e., at the end of collection.

Nei’s genetic distance is a measure of the overlap in the genetic content of populations and will be measured at both strain level (a single measure of similarity using ST number) and at allele level (similarity measured across the seven MLST loci) and is used between isolates from pairs of sources and from different periods.

Simpson’s Diversity Index measures the diversity of strains by host.

**Task 02. Attribution of human cases to the sources**

Attribution by microbial sub-typing is a relatively new area of research. The term “source attribution” has been defined as: “…the partitioning of the human disease burden of one or more foodborne infections to specific source, where the term source includes animal reservoirs and vehicles (e.g. foods)” (Pires, Evers et al. 2009).

Furthermore, the microbial subtyping methodology uses the distribution of subtypes in each of the sources and compares this with the distribution found in humans. This can be done in terms of simple proportions (e.g. the Dutch model) or using Bayesian stochastic methods (e.g. STRUCTURE). Currently, there are five
main techniques for attributing disease on a population level using microbial subtyping (EFSA 2010). Three of these methods were used in the current study (Table 2) and are detailed below. Although the attribution statistics are different, the results are consistent, which enhances the robustness of the conclusion that chicken is the principal source of human disease associated with Campylobacter.

**The Dutch Model** (French 2008).

The Dutch model is a straightforward way to estimate the attribution of a particular genotype (e.g. ST) to a reservoir, when the frequency distribution of each type is known for each reservoir. If \( p_{ij} \) represents the frequency of type \( i \) (e.g. ST 19) in source \( j \) (e.g. poultry) then the proportion of attribution of type \( i \) in source \( j \) is given by

\[
\lambda_{ij} = \frac{p_{ij}}{\sum_{j} p_{ij}}
\]

where the summation by \( j \) considers all the reservoirs where data exist (e.g. cattle, sheep, wild birds, chicken, turkey etc.).

When applied at ST level this model does not guarantee that all STs will be attributed to sources. This is because human types that are not found in the animal reservoir cannot be attributed. However, if genetic information exists at multiple loci as in this study, then the Dutch Model can make use of the frequency of each individual allele at each individual locus, and estimate attribution even for STs that are not present in the animal reservoirs. In particular, at allele level the frequencies \( p_{ijk} \) can be calculated for each allele \( a_{ijk} \) of all isolates from the animal reservoirs. Where \( i \) is subtype, \( j \) source and \( k \) the loci number.

The attribution score of bacterial subtype \( i \) in source \( j \) is

\[
\lambda_{ij} = \frac{\prod_{k=1}^{7} p_{ijk}}{\sum_{j} \left( \prod_{k=1}^{7} p_{ijk} \right)}
\]

where \( p_{ijk} = \text{BetaInv}(0.5,0+1,N_{\text{isolates}} + 1) \) if its frequency is zero (BetaInv fn in Excel).

This assumes that we have no prior knowledge of \( p_{ijk} \) and so is maximally noncommittal or conservative.

The Dutch Model does not take into account the uncertainty in the frequency distribution of genotypes. It does not consider any information about the exposure of humans to sources or the viability/virulence of pathogens once they are ingested by humans. In self-attribution model-validation tests, Dutch model has similar probabilities (59.5%) to that of STRUCTURE (65.7%) of correct assignment of an isolate to the host (Table 2), but it fails to consider uncertainty and sample size for data analysis.
**STRUCTURE** (Pritchard, Stephens et al. 2000).

This is a Bayesian clustering model designed to infer population structure and to attribute individuals to population groups. The program can use MLST genotyping data. Each isolate is attributed on the basis of a training dataset consisting of isolates from known populations (i.e. set USEPOPINFO to 1). The algorithm calculates the frequency of each particular sequence type in each population taking into account the uncertainty due to the sample size. Based on these frequencies, the probability to belong to a population group/reservoir is calculated following multiple iterative steps (Markov chain Monte Carlo - MCMC) for the estimation of frequencies. The programme has the option to consider the allele independently (no-admixture model – independent alleles) and starts with equal frequencies for each isolate type. Following an initial number of MCMC burn-in steps (e.g. 1000) further iterations (e.g. 10000) are used for estimation of the probabilities that an isolate belongs to each particular population being considered (e.g. cattle, sheep, chicken, turkey etc.). To enable the largest reference dataset to be used (often datasets are small due to the cost of typing many isolates) only one ST is selected at a time from the unknown dataset by using the jackknife method. This process is repeated to enable multiple estimations of the same sequence type so that uncertainty in the attribution scores can be determined.

STRUCTURE can be used at ST or allele level (Table 2) as it incorporates uncertainty and takes account of sample size. In self-attribution model-validation tests, STRUCTURE consistently gave a higher probability (65.7%) than Asymmetric Island model (54.4%) of correct assignment of an isolate to origin at the allelic level (Table 2). But gives a more realistic estimation of the attribution to a specific reservoir than the Dutch Model. Also, like the Dutch Model, at allele level it can assign human cases that have STs that are not found in the animal reservoirs. However, it is highly time consuming and does not consider any exposure to risk factors or the viability of pathogens.

**The Asymmetric Island (AI) Model** (Wilson, Gabriel et al. 2008).

This model incorporates a Bayesian approach and uses the allelic profile of the sequence subtypes to reconstruct the genealogical history of the isolates. The host populations are considered to exist on separate “islands” (e.g. the sheep island). Mutations and recombination occur on each island. Migrations from between each reservoir (island) into the human population are used to estimate the degree of attribution to each source. This model has previously been applied to MLST data from England (Wilson, Gabriel et al. 2008), Scotland (Sheppard, Dallas et al. 2009) and New Zealand where 56%, 78% and 75% of human cases were attributed to poultry respectively.

The Asymmetric Island model incorporates recombination and mutation, uses MLST data at the allele level and achieves relatively high values for self-attribution. However, the model appears to be complicated and the current explanations of its operation difficult to comprehend. The Asymmetric Island model assigns each human case to the potential source populations on the basis of DNA sequence similarity. By comparing human isolates to a panel of reference sequences of known source (e.g. cattle, sheep, chickens, pigs, wild birds and turkey), each human case can be assigned a probability of originating in each source population. The source attribution probabilities are calculated using a statistical model of the way the DNA sequences evolve in the populations of bacteria. In the statistical model, there are parameters representing the processes
of mutation, DNA exchange between bacteria (recombination or horizontal gene transfer) and zoonotic transmission between populations. These processes lead to differences in gene frequencies between the source populations, facilitating source attribution. The model can be trained, by estimating the parameters exclusively from the sequences of known sources, before using it to calculate source attribution probabilities for *Campylobacter* obtained from human clinical samples. The i-CaMPS 2011-2012 report established that the use of allelic, rather than the coarser ST typing of strains, gave the most useful attributions; and has therefore been employed in this report (Table 2).

**Self-attribution**

Self-attribution or correct attribution is a key performance measure for the models used in this study (Smid, Mughini Gras et al. 2013, Sheppard, Dallas et al. 2009). This is the average percentage accuracy that any given isolate from a reservoir can be correctly attributed back to its own reservoir. This can be performed in a number of ways. By chance you would expect a correct self-attribution of 20% and 14% for 5 and 7 sources respectively. The average correct attribution percentages for each model shows that STRUCTURE-alleles has the highest (~66%) average correct attribution score (Table 2). STRUCTURE-alleles gives output typical for the other tests, whilst for Asymmetric Island (AI) the underlying assumptions and methodology are rather different – and so gives different outcomes. Ranges are reported as between 62-97% for between 5-7 hosts for the Asymmetric Island model (Sheppard, Dallas et al. 2009, Wilson, Gabriel et al. 2008) and 38-70% for STRUCTURE (Sheppard, Dallas et al. 2009). Taking into account all the various models we found that Dutch alleles, STRUCTURE alleles and AI gave the best scores in self-attribution of isolates and so these have all been used to give a balanced overview of source attribution. It is important to note that modelling provides insights into what would ideally happen but it may not reflect exactly what is happening.
Table 1. Host isolate datasets.

<table>
<thead>
<tr>
<th>Host</th>
<th>Collected</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>2010 -12 &amp; 2005 -06</td>
<td>529</td>
</tr>
<tr>
<td>Sheep</td>
<td>2010 -12 &amp; 2005 -06</td>
<td>342</td>
</tr>
<tr>
<td>Chicken</td>
<td>2010 -12 &amp; 2005 -06</td>
<td>659</td>
</tr>
<tr>
<td>Wild Birds</td>
<td>2005 -06</td>
<td>188</td>
</tr>
<tr>
<td>Pigs</td>
<td>2005 -06</td>
<td>40</td>
</tr>
</tbody>
</table>

**Host dataset pre-2012:** Combined 2005-07 Scottish-wide plus 2010-12 Grampian-wide hosts.

**Host dataset post-2012.**

<table>
<thead>
<tr>
<th>Host</th>
<th>Collected</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>2012-2015</td>
<td>257</td>
</tr>
<tr>
<td>Sheep</td>
<td>2012-2015</td>
<td>296</td>
</tr>
<tr>
<td>Chicken</td>
<td>2012-2015</td>
<td>641</td>
</tr>
<tr>
<td>Turkey</td>
<td>2012-2015</td>
<td>118</td>
</tr>
<tr>
<td>Wild Birds</td>
<td>2012-2015</td>
<td>6</td>
</tr>
<tr>
<td>Pigs</td>
<td>2005-2009</td>
<td>197</td>
</tr>
</tbody>
</table>

Combined 2005-07 Scottish-wide (CAMPS), 2010-12 Grampian-wide hosts (i-CaMPS-1,-2). Host dataset post-2012 (i-CaMPS-3)

Table 2. Molecular attribution models used.

<table>
<thead>
<tr>
<th>Model</th>
<th>Genetic unit of assessment</th>
<th>Average correct self-attribution (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ST</td>
<td>Allele</td>
</tr>
<tr>
<td>Dutch proportional</td>
<td>x</td>
<td>√</td>
</tr>
<tr>
<td>STRUCTURE</td>
<td>x</td>
<td>√</td>
</tr>
<tr>
<td>Asymmetric Island</td>
<td>n/a</td>
<td>√</td>
</tr>
</tbody>
</table>
3. Results and Discussion


*Campylobacter in food and animal reservoirs and humans*

Amongst the food and animal isolates (Table 3, Figure 7), there continues to be strains characteristic of each host species, with other strains being generalists. The prevalence of isolation of *Campylobacter jejuni* and *C. coli* since 2005 to date is summarised in Table 3. The prevalence of *Campylobacter* in food and animal reservoirs has changed over time which might be due to subtle changes in laboratory protocols or to differing staff, or to real changes over time.

**Cattle**

The prevalence of *Campylobacter* in cattle has broadly remained the same since the i-CaMPS -1, -2 (2010-12) study, but has more than doubled since the CaMPS (2005-07) study. The prevalence of *Campylobacter* in this study was 51% (Table 3). The most prevalent strains belonged to STs 42, 827, 19, 61 and 21 (Figure 7). This is consistent with the previous i-CaMPS-2 report. Other species isolated from cattle faeces were *Campylobacter hyointestinalis* (prevalence: 6.5% (33/506)) and *Campylobacter fetus* (prevalence: 4.9% (25/506)).

Previous studies have shown that the three most common clonal complexes associated with strains isolated from cattle in the UK belonged to STs 21, 42, 61 and 45 (French, Barrigas et al. 2005, Kwan, Birtles et al. 2008). ST21, ST42, and ST61 clonal complexes have also been associated with strains isolated from cattle and human in the US (Sanad, Kassem et al. 2011). Earlier studies have also shown that isolates from the ST45 complex were much more frequently isolated from environmental water sources (Bronowski, James et al. 2014). In our study 9 STs belonging to clonal complex 45 were either from cattle or sheep (5 cattle and 4 sheep). ST827 and ST19 have been associated with cattle and human in both North-eastern and South-western regions in Scotland (Rotariu, Dallas et al. 2009). In this current study we found that ST19 occurs 40 times in cattle and ST827 occurs 45 times indicating that these sequence types are very specific to cattle host reservoir.

**Sheep**

The overall *Campylobacter* prevalence in sheep was 76%. The prevalence in sheep has increased by 15% since the i-CaMPS -1, -2 (2010-12) study and has more than doubled since the CaMPS study (2005-07). The STs 19, 21, 61, 827 that were found in significant numbers in cattle were also observed in sheep isolates, with ST42 found in lower numbers in sheep. STs 206, 262 and 825 were found to be associated with sheep host reservoir (Figure 7).

Isolates belonging to ST 19, 21, 42, 61, 206 have been isolated from sheep reservoirs in the UK (Rotariu, Dallas et al. 2009, Senior, Bagnall et al. 2011, Kwan, Birtles et al. 2008, Colles, Jones et al. 2003, Grove-White, Leatherbarrow et al. 2011). A previous study has reported the association of isolates belonging to STs 262 and 206 with abortion in sheep (Wu, Sippy et al. 2014). STs 825 and 827 have also been found in chickens but in our study, ST827 was the most dominant sequence type associated with cattle or sheep in Scotland (Mughini Gras, Smid et
al. 2012) (Figure 7). ST825 isolates were found to be highest in sheep with negligible contribution by other sources.

Pigs
The *Campylobacter* prevalence in pigs in the current study was 31% which was similar to the 27% in the CaMPS study (2005-2007), all of the isolates being *C. coli* (Table 3).

*Campylobacter coli* is generally accepted as a common inhabitant of the intestinal tract of swine and high prevalence of *C. jejuni* may be found in pigs raised on specific farms (Harvey, Young et al. 1999). Although in this study *C. jejuni* was not found and MLST types in pig isolates were not in the 30 most common strains found in other host reservoirs throughout this research, which indicates that the pigs reared in Grampian are not a major cause of campylobacteriosis in Scotland. ST854 was found to be the most common strain isolated from pigs. Previous reports from the US and Switzerland associate ST854 with pigs and it has also been associated with both pigs and broilers in Denmark (Litrup, Torpdahl et al. 2007, Thakur, Gebreyes 2005, Kittl, Heckel et al. 2013).

*Campylobacter lanienae* was the second most common species isolated from pigs, the prevalence for which was 25% (92/361).

As all of the *Campylobacter* isolated were either *C. coli* or *C. lanienae*, we can conclude that these strains isolated from pigs in Grampian are most likely to be host specific.

The low prevalence of *C. jejuni* and *C. coli* from pigs was tested to confirm this was not due to the isolation method. Chromogenic plates from two companies (E&O, Oxoid) were tested for selectivity for *Campylobacter jejuni* or *C. coli* from the pig samples. But this showed no improvement in isolation of *C. jejuni* over charcoal-cefoperazone-deoxycholate agar plate (mCCDA), and so mCCDA continued to be used. Neither of these modifications improved the selective and preferential recovery of *Campylobacter jejuni* or *C. coli*. Recovery of pig isolates from -80°C archive was also problematic, however enrichment broth culture allowed recovery of a few that would otherwise have been lost. In general, the pig isolates were difficult to grow and at times the isolation of DNA was problematic. In part, this poor recovery of archived isolates may be due to the very high proportion of (latex positive) *Campylobacter* isolates which at sequencing proved not to be *C. jejuni* or *C. coli* but *C. lanienae*. The use of chromogenic media and the ambiguous test results using the 1% glycine test (negative for *C. lanienae*) did not help in the detection of *C. jejuni* or *C. coli*.

Chicken
In the current study, the prevalence of *Campylobacter* in chicken was 82% (Table 3). This was a 10% decrease compared to i-CaMPS-1, -2 (2010-12) study.

It was observed that ST50 was one of the most dominant sequence types which has been increasing since 2011 (i-CaMPS-2). Isolates belonging to ST48 were not found to predominant in the previous studies (CaMPS, i-CaMPS-1,-2; 2005-12) but have risen to 2nd position in the current study. This indicates that strain prevalences change overtime and could indicate that certain strains are better at colonising.
It is important to note that strains belonging to ST48 and ST50 have been isolated from humans as well as poultry production chains in New Zealand and the Netherlands (Mullner, Collins-Emerson et al. 2010, Smid, Mughini Gras et al. 2013). Previous studies have reported that strains belonging to ST48 have been isolated from humans in the UK and from chickens (Cody, McCarthy et al. 2012, Dingle, Colles et al. 2002). A study commissioned by DEFRA in 2006 (OZ0613) reported that ST50 was one of the most abundant STs isolated from broilers in UK abattoirs. There were no recent reports indicating that ST50 strains are now more common in chickens in other parts of the UK. Although this study found that strains belonging to ST50 were frequently isolated from chickens tested in this study.

ST5136 was undetected during the 2005-2007 study and was first recorded in 2010 from a stool sample in Oxford. This strain is now the 3rd most commonly found strain in chickens, a position it has maintained since 2011 (i-CaMPS-2). ST5136 is almost exclusive to chicken but now is also seen in turkey; it was the fifth most common ST observed in our clinical isolates (Figure 7). The 2010-2011 study found ST5136 in 26 clinical (4%) and 14 chicken (8%) samples. At that time, chicken isolates originated from a single company but from three different plants throughout the UK. From 2012-15, it was recovered from 18 different abattoirs. STs 45, 51 and 257 were the 4th, 5th and 6th most common STs isolated from chickens in this study. Earlier studies have shown that isolates belonging to ST45 were much more frequently isolated from environmental water sources (Bronowski, James et al. 2014). However, in our study ST45 is clearly associated with chickens, turkey and humans (Figure 7). ST257 has been observed in broilers across UK but only one isolate belonging to ST51 has been reported, in a recent study in UK (Colles, McCarthy et al. 2015, Cody, McCarthy et al. 2012). The sequence types ST5, ST21, ST22, ST42, ST464, ST1044 found to be in significant numbers during CaMPS, i-CaMPS-1,-2 (2005-2012) have decreased in abundance in the current study.

**Turkey**

The overall prevalence of *Campylobacter* in turkey in the current study was 39% (Table 3). This was because the direct counts for turkey were very low both by direct plating and by enrichment. This much lower prevalence in turkey than chickens was unexpected given the commonality of the chicken and turkey production systems. The prevalence in turkey has been correlated with both the age and sex of the birds. Higher prevalence of *Campylobacter* in female turkey has been reported compared to males with females being positive at the second week of age whereas males turn positive a week later (El-Adawy, Hotzel et al. 2012). Prevalence of *Campylobacter* in turkey has not been widely reported from abattoir, with both high and low rates being reported. This may relate to the slower line speeds in turkey production system or practices employed in processing turkey meat at specific abattoirs (Atanassova, Reich et al. 2007, Perko-Makela, Isohanni et al. 2009). At retail, where all samples were collected, there is a greater preponderance in turkey products of skin-off and this may have lowered the prevalence. Our findings concur with a previous study reported by researchers from Denmark, where higher rates of positivity were observed in neck flaps (23 positive/41) than from turkey meat (11 positive/61) (Borck, Stryhn et al. 2002). Their study also found that immunological assays such as EiaFoss and Minividas were simple and fast methods for detecting *Campylobacter* in turkey both from faecal material and environmental samples, thereby providing more accurate results than the routine culture methods. Turkey meat retail products tested in
Germany showed a prevalence of 34% for *Campylobacter*, which is similar to our study (Atanassova, Reich et al. 2007). A Finnish study also reported a similar rate (28%) of *Campylobacter* in the final meat products (Perko-Makela, Isohanni et al. 2009). There was also a higher proportion of *C. coli* to *C. jejuni* than in chickens. A study on the prevalence of *Campylobacter* from turkey has not been performed previously in the UK and this study is the first to include turkey samples in order to understand its role in human campylobacteriosis. The STs that were most frequently found in turkey were broadly the same as chicken (STs 50, 5136, 45 and 51) except STs 990, 583 and 137 which were found almost exclusively in turkey, having not been reported elsewhere. Our data indicates that some strains do overlap between turkey and chicken but campylobacteriosis due to turkey strains is low due to a number of factors: low loads of *Campylobacter* in turkey meat samples that were processed and because the Agriculture and Horticulture Development Board (AHDB) market intelligence data based on DEFRA, HMRC and ONS data suggests that chicken meat is more popular than turkey with per capita consumption of 30kg/individual (Clements 2015) compared with turkey at 3kg/capita consumption. It is also worth noting that turkey meat production has halved since 2000 (Clements 2015). The longer storage period between slaughter and consumption may have an impact as *Campylobacter* loads will decrease over time on the dead bird. Hence, although turkey strains are similar to chicken strains, according to our study there is evidence to suggest that it is not an important source of *Campylobacter* infection in Scotland.

**Humans**

Amongst the clinical isolates (Figure 8), there continues to be variation in the abundance of the different strains detected over time. In the previous studies we observed that ST50 dominated. ST122 has continued to increase dramatically in proportion since it was first detected in appreciable numbers in 2012 (14th most prevalent). Declining STs include ST21 (4th most prevalent) which until 2012 had consistently been the most prevalent clinical strain. Other STs such as ST53 and ST19 have also declined recently. Overall, in the clinical population these trends seem to indicate a succession of strains which span several years. The clinical STs 21, 257, 48, 45, 50, 51 and 5136 were found to be the predominant STs among all isolates sampled (Figure 7). Isolates belonging to the ST21 clonal complex, associated with humans, have also been observed in a previous study in Finland (de Haan, Kivisto et al. 2010). The ST-21 and ST45 clonal complexes were often regarded as the most common lineages causing human disease (Dearlove, Cody et al. 2015). STs 257, 48 and 45 clonal complexes were found to be associated clinical *Campylobacter* isolated in Oxfordshire, UK (Cody, McCarthy et al. 2012). ST5136 was undetected during the 2005-2007 study and was first recorded in 2010 from a stool sample in Oxford. In the current study it was the 5th most abundant strain in clinical cases.
Table 3. Numbers of specimens collected, *Campylobacter* spp. isolated, complete MLST 7-locus genotypes obtained.

<table>
<thead>
<tr>
<th>Source</th>
<th>Specimens collected</th>
<th>Putative <em>Campylobacter</em> positive specimens</th>
<th>Strain typing</th>
<th>Study prevalence</th>
<th>Overall <em>C. coli</em> &amp; <em>C. jejuni</em> prevalence from previous studies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>overall</td>
<td>CaMPS, 1, 2 (2005-2006)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C. jejuni</td>
<td>CaMPS, 2 (2010-2012)</td>
</tr>
<tr>
<td>Cattle</td>
<td>506</td>
<td>350</td>
<td>22</td>
<td>71</td>
<td>223</td>
</tr>
<tr>
<td>Pig</td>
<td>361</td>
<td>278</td>
<td>45</td>
<td>121</td>
<td>0</td>
</tr>
<tr>
<td>Sheep</td>
<td>392</td>
<td>317</td>
<td>12</td>
<td>9</td>
<td>196</td>
</tr>
<tr>
<td>Chicken</td>
<td>785</td>
<td>750</td>
<td>40</td>
<td>69</td>
<td>618</td>
</tr>
<tr>
<td>Turkey</td>
<td>301</td>
<td>126 (7)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6</td>
<td>9</td>
<td>102</td>
</tr>
<tr>
<td>Wild Bird</td>
<td>84</td>
<td>21</td>
<td>2</td>
<td>13</td>
<td>6</td>
</tr>
<tr>
<td>Human</td>
<td>2393</td>
<td>2393</td>
<td>104</td>
<td>11</td>
<td>2064</td>
</tr>
</tbody>
</table>

*a*: Total number of *Campylobacter* spp. positive specimens (by latex agglutination test).

*b*: 7 turkey isolates were from APHA.
Population diversity

Rarefaction
The extent to which the isolates from reservoirs in sampling plans identify all of the genotypes was characterised using rarefaction. Rarefaction is a data re-sampling technique that indicates whether sampling of genotypes has reached a plateau or is still rising at the end of collection. A rarefaction curve that has reached a plateau indicates that all genotypes (e.g. MLST) have been sampled whereas an increasing slope indicates that some genotypes remain unsampled. This method assumes that the dataset represents a random sample taken from a closed system characterised by a constant, stable spectrum of types. The rarefaction curves for all clinical, environmental and food sources in 2010-2011 and current study (2012-15) are still rising, even at the maximum sample sizes (Figure 9).

The system being studied is open to immigration (e.g. for human clinical strains there will be immigration by foreign travel) nor is the sampling size sufficiently large to be comprehensive. We can conclude that the clinical and chicken strains have similar levels of diversity and their curves continue to rise as new STs emerge. However, the cattle and sheep strains exhibit less diversity (plateaued curves) than those from retail chicken and human clinical strains. The rarefaction curves for all isolates are rising indicating that for all there are new strain types that would be detected with increased sample size (Figure 9).

Simpson’s Diversity Index
Simpson's Diversity Index is a measure of diversity of strains that takes into account the number of strains present, as well as how common or rare the strains are compared to the population of a given source. A value of zero indicates a population with few strains. A value towards 1.0 indicates a high genetic diversity of isolates. The index quantitates the variety of strain types found in a source (Figure 10). Chicken and turkey isolates and those recovered from cases of human infection both have the largest diversity. The diversity in each source has been constant since 2005 with the exception of a reduced strain diversity in ruminants during the 2010-12 period (Strachan, Rotariu et al. 2013).

Strain distributions across reservoirs
The extent of overlap of strain types between chicken, turkey, cattle, sheep, pigs, wild birds and human reservoirs was explored descriptively by Venn diagrams (Figure 11). Specifically, the relationship of strain overlap between clinical, chicken, turkey was explored, which allowed the aggregation together of the other reservoirs. Of the 432 different STs detected in i-CaMPS-3 there were 117 STs in cattle, sheep, pigs and wild birds, 103 ST in chicken, 37 ST in turkey and 318 ST in humans indicating that there are many ST that are found in several different hosts. There are fifteen different combinations by which these four sources can be grouped together and contain strains that are found uniquely in one group through to the 10 STs that were found common to all four groups (Figure 11(a)). Of the 3,764 isolates mapped to the Venn diagram (Figure 11(b)), over a third mapped to this multi-host group of 10 STs. These 10 STs are found in many reservoirs; they are generalist strains. The implication of this is that there could be a flow of these strains between the reservoirs and so elimination of these strains from other source (e.g. chicken), would not remove the possibility of their reinvasion from the other sources.
This concentration of isolates into comparatively few ST indicates the challenge that is faced when using molecular attribution modelling, which clearly has to include the relative abundance of each ST in the reservoirs to calculate the attribution proportions. Further, within human isolates, 51% of all their STs were only found in human cases and these represented 18% of all human isolates. This is most likely to be a reflection of the very large strain diversities seen in all the sources and in clinical isolates: rare strains are, by their nature, uncommon and so will not be detected readily in more than the one host in which they were first found. The possibility that there is a major but unknown source of Campylobacter which has not been screened in this study is implausible given the extensive epidemiological research which has been carried out around the world.

As a result, the preferred attribution models are based on seven locus definitions of strains. Effectively, an isolate is attributed using seven variables, rather than the one variable of ST, with the likelihood that many of its allelic variants will be seen in other strains. Also of note is the extensive proportion of STs (57%) that are singletons (i.e. a strain or ST represented by only a single isolate) within reservoirs. All groups (except turkey which has fewer isolates) showed similar proportions and since by definition these singleton strains are unique to the host from which they were isolated, this suggests that new strains are likely to be evolving in all of these sources. Figure 11 indicates that there is much more overlap of strains between turkey and chicken than there is with the other tested reservoirs (cattle, sheep, pig, wild bird). This may be a reflection of commonality of production systems and/or greater phenotypic similarity of the two species.

The descriptive strain distributions in the Campylobacter population were also examined using Nei’s genetic distance between isolates from the different sources (Figure 12). Nei’s genetic distance is a measure of the overlap in the genetic content of populations and this was measured at both strain level (a single measure of similarity using ST number) and at allele level (similarity measured across the seven MLST loci). Nei’s genetic distance provides a measure of the overlap of the distribution of strains between different sources, thus populations with many similar alleles have small genetic distances.

Nei’s genetic distance comparing i-CaMPS-1, -2 (2010–12) with iCaMPS3 (2012–2015) is described in Figure 12 (a) indicates that chicken and sheep populations in the current study were significantly different than i-CaMPS-1 and -2. Standardized genetic distances (d1) between clinical vs. other host and chicken vs. other hosts are shown in Figure 12 (b) and illustrate that for chicken isolates there are significant differences in their genetic distance to the isolates from all other hosts. Turkey isolates being most similar and pig isolates being more distinct. The greatest similarity of chicken isolates was to clinical isolates suggesting a linkage between both of these. The genetic distance of clinical isolates to other hosts strongly paralleled that of chicken isolates with turkey being next most similar and pigs least similar.

Comparison of the genetic distance of this study’s isolates (2012-2015) to those from 2010-2012 (Figure 12 (a)) revealed that chicken isolates were significantly more diverse from each other than cattle, sheep or pigs. This implies that there is a more rapid turnover of chicken strains with time compared to other sources. It suggests that attribution studies should make greater efforts to utilise contemporaneous chicken isolates than may be required for isolates from other sources.
The strains of *Campylobacter* present in clinical, environmental and food sources in Scotland represent an extremely large pool of strains that is continually being augmented: internally by mutation and recombination (French, Barrigas et al. 2005) and externally by strain input from human travel and migrating wildlife. Also there is evidence that certain mutations in genes, such as *gyrA* (quinolone resistance determining region) have shown to confer selective advantage by positive selection in the process of evolution (Luo, Pereira et al. 2005).

**Other bacterial species identified**

Other bacterial species such as *Arcobacter butzleri*, *Lactobacillus salivarius*, *Acinetobacter sp*, *Campylobacter lari*, *Campylobacter hyointestinalis*, and *Campylobacter lanienae* were isolated in the samples collected from the animal reservoirs. This has been a major issue in pigs where almost half of isolates failed to provide complete STs after running through the bioinformatics pipeline. Other species such as *C. lari* were isolated from 6 human, 1 chicken and 1 pig sample and *C. upsaliensis* was isolated from one human sample. Some of the ‘putative’ *Campylobacter* positive samples when whole genome sequenced belonged to other families related to different genera e.g. *Acinetobacter* spp, *Arcobacter* spp, *Campylobacter* sp nov *molothri*. Strains of *Campylobacter*, *Arcobacter* and *Acinetobacter* isolated in this study are being classified to species level using several bioinformatics tools (Lagesen, Hallin et al. 2007, Richter, Rossello-Mora 2009), and further analyses are on-going at the Universities of Aberdeen and Utrecht and at the United States Department of Agriculture- Agricultural Research Service. This work is at the boundaries of taxonomic knowledge and is continuing.

*Campylobacter fetus* have been associated with abortion of fetus in cattle and sheep (Wagenaar, van Bergen et al. 2014). It is possible that selective isolation methods used in food microbiology are not suited for its detection. People at higher risk include elderly and immunocompromised individuals and those with occupational exposure to infected animals (Wagenaar, van Bergen et al. 2014). *C. hyointestinalis* and *C. lanienae* have been isolated from pigs where the former is associated with intestinal disorders such as porcine proliferative gastroenteritis and diarrhoea in pigs and the latter has been isolated from healthy pigs (Gorkiewicz, Feierl et al. 2002, SASAKI, FUJISAWA et al. 2003). Other than pigs, *C. hyointestinalis* has been found in a wide range of host species including cattle, deer, hamsters and humans where it is known to cause different types of infections but the pathogenesis remains unclear (On, Bloch et al. 1996). *C. lari* strains have been isolated from intestinal contents of seagulls and other animals and from river water and shellfish (Debruyne, On et al. 2009). In humans it has been isolated from faeces of immunocompromised patients (Martinot, Jaulhac et al. 2001). *Campylobacter upsaliensis* was first discovered in dogs and since then has been acknowledged as an enteropathogen humans (Bourke, Chan et al. 1998). Other less common species of *Campylobacter* that are known to cause mild to severe infection in varied host species include *C. rectus*, *C. gracilis*, *C. showae*, *C. hominis*, *C. concisus*, *C. mucosalis*, *C. sputorum*, *C. helveticus* and *C. insulaenigrae* (Maher, Finnegan et al. 2003, Macuch, Tanner 2000). A phylogenetic tree has been plotted by extracting 16S rRNA sequences of the Epsilon-proteobacteria group which include *Campylobacter* and other related organisms by the maximum likelihood method (Figure 13). This method searches for the tree with the highest probability or likelihood.
Figure 7. ST Abundance in Clinical, Food and Environmental Isolates in iCaMPS3.

Error bars are 95% CI calculated by bootstrapping.
Figure 8. ST Abundance in clinical isolates by study periods.

Number of clinical isolates represented in each graph: 2001 (n=172); 2005-2007 (n=1452); Apr 2010-Mar 2011 (n=694); Apr 2011-Mar 2012 (n=588); Apr 2012-Mar 2013 (n=812); Apr 2012-Apr 2015 (n=2278). Error bars are 95% CI calculated by bootstrapping.
Figure 9. Rarefaction (saturation analysis).
Figure 10. Simpsons Diversity index of isolates in i-CaMPS3

Error bars are 95% CI calculated by bootstrapping.
Figure 11. Abundance of ST and isolates in different sources.

(a) Distribution of all ST identified in i-CaMPS3 stratified by which sources they were identified in. Strains common to only Chicken-Clinical are indicated by the dashed line; there were no strains common to only Turkey-Cattle, sheep etc.

(b) Distribution of all isolates in each source group (indicated as number of isolates) and coloured as proportion of the total isolates in that source group (red: >30%; orange: >10%; green: <10% of isolates).
Figure 12 (a) Nei’s genetic distance comparing period i-CaMPS 1 - 2 (2010–2012) with iCaMPS3 (2012-2015) (b) Clinical v/s other hosts and chicken v/s other hosts for i-CaMPS-3 (2012-2015).

(a) Datasets are normalised to a common population size (100 isolates) to allow comparison within and between sets. Error bars are 95% CI calculated by bootstrapping. * indicates significant difference.

(b) Error bars are 95% CI calculated by bootstrapping.
Figure 13. Phylogenetic tree based on 16S rRNA sequences of Epsilon-proteobacteria group.

Phylogenetic tree (the branch length is proportional to the number of substitutions per site).
3.2. Objective 3, Task 02. Attribution of human cases to the sources

Reference isolate datasets

The 7 locus MLST typing data for the isolates from chicken, turkey, cattle, sheep, pigs and wild birds is used to determine the proportions of clinical isolates that originate from these different reservoirs. Studies at the University of Aberdeen have accumulated isolates over some ten years and from several different sources. Attribution analyses require representative isolates from appropriate reservoirs which are preferably contemporaneous with clinical isolates. i-CaMPS-3 has collected isolates from cattle, sheep, chicken, turkey and pigs. Wild bird isolates were not obtained in sufficient quantity and so these isolates were taken from the 2005-07 CaMPS study (Table 1).

Increasing the number of isolates by including those from previous study periods, would lead to larger datasets, however this can only be appropriate if the temporary separate collections are genetically homogeneous. This was determined with Nei’s genetic distance. This test indicated that the chicken and the cattle datasets from i-CaMPS-1 and -2 compared to i-CaMPS-3 were significantly different (p<0.05) (Figure 12(a)). The genetic distance between chicken and turkey in the current study was 0.2539 (95% CI 0.2087 - 0.3056) and significantly different from the genetic distance obtained from the mixed population (p-0.0075), implying that populations are different and therefore were not combined for attribution purposes. Table 1 summarises the reservoir isolates used in this study.

Self-attribution and model choice

The molecular attribution models used in this study were Dutch proportional, STRUCTURE and AI, in all three cases employing the more resolving allelic typing data. It is important to note that no model can be regarded as the best choice because each model has its own merits and demerits. To highlight this we have performed self-attribution model validation tests.

Since Nei’s genetic distances were always smaller when determined using the allele rather than the ST datasets the attribution analyses utilised the more refined allele level data (Table 2). The self-attribution scores for the individual isolates stratified by host of origin (Figure 14) indicates the spread of scores obtained. In Figure 14 various colours indicate a particular source. For various sources we can draw the following inferences as indicated below:

Chicken: We can see that STRUCTURE and Dutch models wrongly attribute some of the chicken isolates to turkey and sheep. This is seen to a lesser extent in Asymmetric island model.

Turkey: The Asymmetric Island model is not good at self-attributing turkey isolates but STRUCTURE and Dutch perform well.

Pigs: STRUCTURE and Dutch models correctly attribute pig isolates, Asymmetric Island model less well.

Wild birds: The self-attribution by STRUCTURE and Dutch models is slightly better than the Asymmetric Island model.
Sheep: Although a slight mix of wrongly attributed cattle and chicken isolates can be seen in attributing sheep by the Asymmetric island model, the other two models misattribute to pigs.

Cattle: STRUCTURE, Dutch and Asymmetric Island models all have similar self-attribution to cattle isolate, with the main confounding source being sheep. Although wrong, self-attribution by pigs and turkey is very low in Asymmetric island model.

Summarised self-attribution scores for each host varied between approximately 40% to 99% (Figure 15). Overall, STRUCTURE gave the highest scores for self- attribution and Asymmetric island model the worst (Table 2). All the models had a high percentage of correctly attributing bird and pig isolates to their respective reservoirs.

**Source attribution**

Based on the three models (STRUCTURE, Dutch and Asymmetric Island) source-attribution (Figure 16) scores for clinical isolates in Grampian ranged from:

- 55% to 75% for chickens
- 10% for cattle
- 10% to 22% for sheep
- 0% to 8% for pigs
- 4% to 8% for wild birds

Retail chicken sources were attributed to the majority of cases of campylobacteriosis in Grampian. Cattle and sheep each contributed some 10-22% of all cases depending on the model. Pigs and wild birds had the smallest contribution to the burden of campylobacteriosis in Grampian irrespective of the model used for analysis.

This is the first study to review turkey as a potential source of *Campylobacter*. The impact of this is complex. For there to be “new” attribution to turkey there must be an equivalent decline in attribution to other hosts, and this impact is apparently variable depending on the attribution models (Figure 16); overall the turkey attribution is 15-25% of cases (appendix Figure 1). Although there is much strain overlap for chicken and turkey isolate (Figures 11, 12), the self-attribution tests for turkey isolates were poor (appendix Figure 2). The prevalence of *Campylobacter* in turkey is less than half that in chicken (Table 3) and consumption of turkey meat is many fold less than that of chicken meat. Whilst the aggregation of chicken and turkey together as ‘poultry’ would seem to offer a useful interpretation, this was not done because although the population of strains in chicken and turkey are similar, there is a statistical difference between them (p<0.005). Further, to support our claim that turkey does not play a major role in campylobacteriosis, we observed that there was no spike in *Campylobacter* associated cases during the festive season (December), when turkey consumption is generally high (Figure 17).

Notwithstanding the above caveats, no change in attribution over time in the relative importance of different sources was observed (Figure 18, 19) over the study periods from 2005-2015. There is a continuing domination of evidence pointing to retail chicken as the primary source of human infection. This is not
unexpected since although there have been interventions introduced by the poultry industry during the time of this study, they have not been widely implemented and there has been no decrease in the number of clinical cases of campylobacteriosis. The temporal attribution with the inclusion of turkey is shown in Figures 20 and 21.
Self attribution of every source isolate by Dutch (a), STRUCTURE (b) and Asymmetric Island (c) models. Isolates are displayed in columns and grouped by the source species from which they had been isolated (listed along the x-axis). For each isolate, its attribution score for each source is calculated using the remaining (N-1) isolates. And the scores graphically displayed by colour along the y-axis.
Figure 15. Self-attribution (correct attribution) of animal isolates by Dutch, Structure and AI models.

Confidence of correct attribution is assessed using self-attribution tests, that is the probability that the model will correctly assign an isolate to the source from which it was actually isolated. By chance for five hosts, there is a 20% likelihood that an isolate would be correctly attributed, and this threshold is indicated by the dashed line. Error bars are 95% CI calculated by bootstrapping.
Figure 16. Source attribution of Grampian clinical isolates in i-CaMPS3 (2012-2015).

Source attribution of clinical isolates in iCaMPS3 using Source Reservoir Dataset. Error bars are 95% CI calculated by bootstrapping.

Figure 17. Monthly attribution to turkey.

Proportional attribution to turkey sources by month. Red dots indicate December.
Figure 18. Attribution to five potential host reservoirs of clinical *Campylobacter* cases in Grampian per month by (a) STRUCTURE with alleles Model, (b) Asymmetric Island Model

(a)

Graph stacked to total number of clinical cases per month. Attribution based on hosts using Host Dataset pre-2012 is displayed to the left of the vertical bar, with distribution based on hosts using Host Dataset post-2012 displayed to right.

(b)
Figure 19. Attribution to five potential host reservoirs of clinical *Campylobacter* cases in Grampian per month by (a) STRUCTURE with alleles Model, (b) Asymmetric Island Model (stacked to 100%).

Graph stacked to total number of clinical cases per month. Attribution based on hosts using Host Dataset pre-2012 is displayed to the left of the vertical bar, with distribution based on hosts using Host Dataset post-2012 displayed to right.
Figure 20. Attribution to six potential host reservoirs of clinical *Campylobacter* cases in Grampian per month by (a) STRUCTURE with alleles Model, (b) Asymmetric Island Model

Graph stacked to total number of clinical cases per month. Attribution based on hosts using Host Dataset pre-2012 is displayed to the left of the vertical bar, with distribution based on hosts using Host Dataset post-2012 displayed to right.
Figure 21. Attribution to six potential host reservoirs of clinical *Campylobacter* cases in Grampian per month by (a) STRUCTURE with alleles Model, (b) Asymmetric Island Model (stacked to 100%).

Graph stacked to total number of clinical cases per month. Attribution based on hosts using Host Dataset pre-2012 is displayed to the left of the vertical bar, with distribution based on hosts using Host Dataset post-2012 displayed to right.
3.3. The sources of human campylobacteriosis in Grampian

Source attribution

Figure 16 shows the attribution of clinical isolates to the sources of cattle, sheep, chicken, wild birds and pigs using the three models (STRUCTURE, Dutch and AI) for which self-attribution has been performed as discussed above. Attribution to sources inclusive of turkey have not been performed, primarily to give consistency of analysis with previous studies, but also because the likelihood of exposure to turkey is considered low as mentioned previously.

In the current study neither pigs nor wild birds in Grampian contributed significantly to the burden of campylobacteriosis; together contributing less than one tenth of cases (Figure 16). Cattle and sheep attributed cases comprised just under a quarter of all cases and chicken contributed two thirds. The source attribution modelling allocated clinical isolates to the following reservoirs: chicken 55-75%, cattle 10%, sheep 10-22%, pigs 0-8% and wild birds 4-8% (Figure 16). It is clear from the analysis that the highest attributions were to chicken; sheep and cattle being secondary and other sources (wild birds, pigs) having a less important role.

Age stratified attribution

Stratifying the attributed source of a case’s isolate by the patient’s age indicated that attribution of clinical isolates from Grampian to the chicken host reservoir was highest, followed by attribution to cattle and sheep reservoirs (Figure 22). For pigs and wild birds the burden is constant with age (Figure 22). An age dependent increase in attribution to retail chicken sources at the expense of ruminant sources had been observed in previous i-CaMPS studies. However, this was not observed in the current study. The confidence intervals in all of these studies are large and preclude significant determination of trends.

Urban-rural stratified analysis

The current study and previous studies show that the incidence of campylobacteriosis for cases living in Aberdeenshire was higher than that in Aberdeen city (Figures 23, 24).

The association between infection with Campylobacter and living in a rural area (Aberdeenshire) as opposed to an urban area (Aberdeen) has been reported in a number of studies (Strachan, Gormley et al. 2009). A study conducted in Grampian (2000 to 2006) found cases of campylobacteriosis in young children (< 5 years) living in rural areas were more likely to be attributable to ruminant and other avian sources compared to children in urban areas for which chicken was the major source of infection (Strachan, Gormley et al. 2009). According to a previous study, the risk of infection was found to be higher in people living in Aberdeenshire (incidence=201/100,000) as opposed to Aberdeen city (incidence=157/100,000) [pages 25-26] (Smith-Palmer, Cowden et al. 2010). A Canadian study carried out in the Manitoba province showed population groups in rural areas, employed in agricultural occupations and living in geographic areas with exposure to high levels of animal densities had higher rates of Campylobacter
infection; between two to three times higher than in lower risk areas (Green, Krause et al. 2006).

Figure 23 (i-CaMPS-3) and Figure 24 (i-CaMPS-2) illustrate the partitioning of cases by three criteria: “chicken” vs “non-chicken” attributed; urban vs rural residence and age. Aggregated age bands were selected to maximise the number of isolates in each group whilst still stratifying into epidemiologically useful groups. In the previous study, there was no difference in incidence between “chicken attributed” and “non-chicken attributed” cases at the level of each individual age group in the urban population (Figure 24). In the current study, overall ages, for both chicken attributed and non-chicken attributed cases the incidence is higher in the rural population than the urban but it is not statistically significant for most of the age groups as error bars overlap. An increased incidence of non-chicken attributed cases for 0-4 and 5-14 age groups was observed (Figure 23). It is clear that rural children were more likely to have a non-chicken attributable strain as compared to urban children (Figure 23). Previous reports also suggest that private water supplies and contact with farm animals are risk factors for campylobacteriosis in the rural environment (Smith-Palmer, Cowden et al. 2010).

The questionnaire

Since January 2011, a three page questionnaire has been sent to all campylobacteriosis cases under Grampian Health Board to request information on their lifestyle and exposure to potential sources of Campylobacter. Over the 51 months prior to March 2015, 1,251 questionnaires were returned; a response rate of 38%, which is considered to be high for such studies (Schwille-Kiuntke, Enck et al. 2011) (Table 4). Questionnaires (n=1046) were linked to the corresponding Campylobacter isolate. This linkage permitted comparison of patient characteristics and behaviours to the genotype of the infecting organism. Of the 1,046 cases who returned a questionnaire, the proportion from woman was comparable to the regional average (52% vs. 51%) (Section 4.5 in the link: www.nrscotland.gov.uk/files/statistics/population-estimates/mid-2013/html/mid-2013-population-estimates-administrative-areas.html ).

The age of respondents ranged from 0 to 93 years and the mean time between the onset of illness and completing the questionnaire was 15d (range 2-75d). To maximise the statistical power of analyses, attribution rather than ST was used. This was done because the latter comprises too many divisions.

Cases hospitalised and severity of symptoms

Although around a million people suffer a foodborne illness, 20,000 people receive hospital treatment each year (FSA 2011). The reported highest risk of severe symptoms of campylobacteriosis is observed in young children (<5 years), the elderly (over 65 years), pregnant women and immunocompromised hospital patients (FSA 2011). Diarrhoea and abdominal pain are the most common symptoms and the vast majority of cases are mild (Altekruse, Stern et al. 1999).

Based on 895 case questionnaires for which the duration of symptoms was known, a total of 466 cases completed the questionnaire whilst the symptoms were ongoing (Figure 25). For 72 cases the duration of illness could not be estimated because respondents did not provide all dates. The duration of symptoms was typically 6-8 days across all age groups (Figure 26). Abdominal pain was reported
in virtually all the cases (91.6%) with incidence of bloody stools (63.5%) and vomiting (67.5%) being more prominent in 0-4 and 5-14 age groups (Figure 27). Hospitalisation was common across the majority of age groups with the exception of under 5s. The proportion of cases was slightly higher in the 5-14 age group (Figure 27). The mean number of days of hospitalisation was 4 days with the exception of the 65+ age group who reported a mean of 6 days in hospital (Figure 27). The fact that 9% (78/840) of cases in this study were hospitalised highlights the potential seriousness of infection and the considerable resultant social and economic burden. These findings concur with a previous study where researchers found that 10% of patients with campylobacteriosis were hospitalised (Tam, Rodrigues et al. 2012).

**Household outbreaks**

Most cases of *Campylobacter* infection are generally regarded as sporadic with outbreaks being rarely reported (Tam, Rodrigues et al. 2012). ObsSurv only captures information on general outbreaks and not on single household outbreaks. A case reporting household members with similar symptoms does not always mean that the individuals were also infected with *Campylobacter*, as the symptoms could be due to another pathogen or even due to a non-infectious aetiology. There were very few cases who reported that others in the household also became ill (6.4%, Table 4) which perhaps suggests that successful infection is a complex process requiring not just exposure to the organism (contact with animals, handling raw chicken), but also that the organism is in a pathogenic state (passage through humans seems to render the organism less pathogenic) and that the individual is susceptible (through prior exposure)(Kim, Artymovich et al. 2012).

**Foreign Travel and Overnight stay**

In the current study 19% of the cases were reported to have an overnight stay abroad 14 days before feeling unwell. In our study we observed that, Thailand (0.011487) and Turkey (0.005776) had the greatest rate/travellers ratio (number of cases ÷ number of passengers) whereas USA (0.000173) and Germany (0.000166) had the lowest. Very few (n=17) of travel cases declared contact with animals whilst abroad. The STs 47, 48, 122, 148, 233, 464, 508, 607, 883, 2258 and 5480 were found to be associated with these cases. All sequence types except STs 2258 and 5480 indicate that *Campylobacter* infection could be from a cattle reservoir. Strains belonging to ST2258 have been isolated from chicken in Belgium, whereas there are no reports on ST5480 (Habib, Miller et al. 2009). There were no dominant STs in all 197 cases although ST21 which is often associated with cattle reservoir was found to be associated with 5.1% of travel cases. ST883, 464, 572 attributed to chicken were mainly associated with travel. These STs have not been in anyway identified as destination associated STs.

The level of risk for travel-related campylobacteriosis appears to be associated with the travel destination. A meta-analysis in 2009 showed that the locations with the highest levels of risk are Southeast Asia (32.4%; 162/500 cases), South Asia (7.8%; 39/499 cases), Africa (4.6%; 54/1,177 cases), and Latin America (2.5%; 51/2,031 cases)(Mughini-Gras, Smid et al. 2014). The risk of travel to Africa and Asia as well as South America in relation to gastrointestinal illness,
has been reported in a number of travel medicine specific studies (CDSC 2003, Greenwood, Black et al. 2008). Previous reports suggest that an overnight stay outside the study area and eating out, positively correlated with each other (Smith-Palmer, Cowden et al. 2010). Cases were significantly more likely to have eaten chicken whilst eating out when compared to controls (OR = 1.52 (1.14 to 2.01), p = 0.0026) (Smith-Palmer, Cowden et al. 2010). The positive correlation between overnight stay outside the study area and eating out was due to both travel within the UK and abroad (Smith-Palmer, Cowden et al. 2010). There could be a possible link between affluence and other exposures including travel and eating chicken outside the home and affordability linked to the number of times fresh chicken is consumed during the week (Smith-Palmer, Cowden et al. 2010).

**Case contact with animals**

In the current study, 8% of cases reported some form of contact with animals and these cases had an increased probability that their isolate was attributable to a cattle or sheep and less likely that it was from chicken (Table 5). Table 5 shows attribution based on STRUCTURE scores for cases that had animal exposure and cases that didn’t have animal exposure. A high score indicates a higher likelihood of getting infected with *Campylobacter* from a specific reservoir. It is clear that cases that had any animal exposure had a greater chance of being infected with ST related cattle, sheep or wild birds (values=0.16, 0.24, 0.11). It can also be seen that cases without any exposure to animals had a greater chance to be infected with a strain common to chicken (value=0.59). This is an independent validation that the molecular attribution concurs with the empirical epidemiology of these cases as has also been reported elsewhere (Mughini Gras, Smid et al. 2012, Smid, Mughini Gras et al. 2013).

The importance of contact with farm animals as a mechanism for contracting *Campylobacter* has been shown in other studies (Gallay, Bousquet et al. 2008, Newell, Fearnley 2003, Stafford, Schluter et al. 2007). For example contact with pets, farm animals and private water supply have been identified as significant risk factors for *Campylobacter* infection (Smith-Palmer, Cowden et al. 2010). Similarly an association with illness in the home and visit to a farm two weeks prior to onset of campylobacteriosis has been reported (Gillespie, O’Brien et al. 2003) as has an association with contact with a pet suffering from diarrhoea (Gillespie, O’Brien et al. 2003).

**Cases with private water supply**

Cases who reported using a private water supply (8.3%) had an increased likelihood of infection by a strain attributable to a ruminant reservoir, however the effect was not found to be significant. Conversely, individuals not on a private water supply were more likely to contract a chicken attributed isolate (Figure 28). In rural areas, it is likely that there will be an association between both private water supply and farm animals (Smith-Palmer, Cowden et al. 2010) and so it is difficult to disentangle these.

There are 8,000 properties with a private water supply in Grampian (Aberdeenshire council ). Individuals are exposed when living in a property with a private water supply or when visiting friends or family or at campsites, guest
houses, etc. (Smith-Palmer, Cowden et al. 2010). The risk of contracting *Campylobacter* infection from private water supplies may be higher in those not normally exposed because of lack of immunity (Havelaar, van Pelt et al. 2009). An Aberdeenshire study comparing mains and private water supplies between cases and controls found 8.0% of cases had a private water supply compared to 2.8% of controls (p<0.001). This highlighted the fact that private water supplies are a significant risk factor for *Campylobacter* infection (Smith-Palmer, Cowden et al. 2010).

**Food handling and eating habits**

In the current study, handling raw chicken (30% cases) in the 5d prior to illness compared to those who did not, did increase the likelihood of infection by a strain attributable to a chicken, however the effect was not significant (Figure 29). 88.7% of cases confirmed that they ate chicken regularly with 82.3% consuming chicken 5 days prior to the illness and 6% consuming chicken liver pâté (Table 4). However, the absence of control groups prevents further analysis of this.

For the UK, an average annual pattern of consumption of chicken is about 30kg/capita (Tam, Larose et al. 2014, Clements 2015). Eating chicken prepared outside the home (37.8% of cases and 28.3% of controls) and attending barbecues or picnics (8.7% of cases and 6.2% of controls) appeared to be a significant risk factor for *Campylobacter* infection (Smith-Palmer, Cowden et al. 2010).

In 2009, the FSA commissioned a consortium to collect baseline quantitative information on the UK public’s reported behaviour, attitudes and knowledge relating to food issues such as food safety and healthy eating (Prior, Phillips et al. 2014). The ‘Food and You’ survey studies 2 and 3 focussed on food safety issues for respondents in England and Wales with additional question modules on healthy eating for respondents in Scotland and Northern Ireland. In study 3, 84% of respondents reported always washing their hands before starting to prepare or cook food, and 97% reported that they did this at least some of the time. The majority of respondents (86%) reported always washing their hands immediately after handling raw meat, poultry or fish. Around half (49%) of respondents said they always used different chopping boards for different foods, whilst 27% said that they never did, similar to study 2. In both studies 2 and 3, 56% of respondents reported using different chopping boards always or most of the time. Approximately 60% of the subjects in study 3 reported that they stored raw meat and poultry on the bottom shelf of the fridge, in line with the FSS and FSA recommended practice.

The ‘Food and You’ Survey (Study 3) reported 36% of subjects who never washed chicken, with 53% reported washing chicken at least sometimes (Prior, Phillips et al. 2014). 54% of the Food and You Survey respondents reported storing raw meat and poultry in their fridge in its packaging which is not as per the FSA recommended practice. These factors, along with splashing of water as a result of washing chicken in sink puts people at risk of infection as a result of cross contamination of surrounding surfaces and utensils which may come into contact with ready to eat food.
Figure 22. Attributed host sources of clinical isolates partitioned by patient age.

(a) Attributed host sources of clinical isolates from Grampian (2012 -15).

(b) Attributed host sources of clinical isolates from Grampian (2005-07 and 2010 -12) and Scotland (2005-06).

Error bars are 95% CI calculated by bootstrapping.
Figure 23. Chicken and Non-Chicken attributed cases from Rural or Urban cases, stratified by age: 2012 -2015.

Isolates attributed to source by STRUCTURE alleles model. Error bars are 95% CI calculated by bootstrapping.

Figure 24. Chicken and Non-Chicken attributed cases from Rural or Urban cases, stratified by age: 2005-2012.

Isolates attributed to source by STRUCTURE alleles model. Error bars are 95% CI calculated by bootstrapping.
Figure 25. Duration of Illness.

Based on 895 cases with known duration of symptoms and for 466 cases where symptoms were still present on the date the questionnaire was completed (thus cases were symptomatic for longer period). Duration could not be estimated from 72 cases.

Figure 26. Duration of Illness by age.

Based on 1006 cases with known duration of symptoms.
Based on cases who returned a questionnaire which could be linked to a clinical isolate and which was typed. Of these cases, those which reported presence or absence of symptoms were: Bloody stools (281 cases positive of 723), Vomiting (344 cases positive of 746), Abdominal pain (859 cases positive of 928), or of being hospitalised (78 cases positive of 840).
Figure 28. Attributed source of isolates from cases who reported using a private water supply.

STRUCTURE scores used.

Figure 29. Attributed source of isolates from cases who reported handling raw chicken prior to disease.

STRUCTURE scores used.
Table 4. Questionnaire summary statistics.

<table>
<thead>
<tr>
<th>Cases with both Typed Isolate &amp; Questionnaire</th>
<th>Jan 2011 – March 2015</th>
</tr>
</thead>
<tbody>
<tr>
<td>Questionnaires returned</td>
<td>1251</td>
</tr>
<tr>
<td>% Return rate (of clinical cases)</td>
<td>38%</td>
</tr>
<tr>
<td>Cases with both Typed Isolate &amp; Questionnaire</td>
<td>1046</td>
</tr>
<tr>
<td>Age range</td>
<td>0-93</td>
</tr>
<tr>
<td>Gender</td>
<td>52% female</td>
</tr>
<tr>
<td>Days between start illness and questionnaire received</td>
<td>2 – 75 (mean 15.0d)</td>
</tr>
<tr>
<td>Did others in household get ill?</td>
<td>6.4%</td>
</tr>
<tr>
<td>Did you have an overnight stay abroad 14 days before feeling unwell</td>
<td>19.0% (197/1036)</td>
</tr>
<tr>
<td>Did you have contact with farm animals 5 days before you were feeling unwell</td>
<td>9.2% (96/1038)</td>
</tr>
<tr>
<td>Private Water Supply?</td>
<td>7.5%</td>
</tr>
<tr>
<td>Did you eat chicken 5 days before you started feeling unwell</td>
<td>82.3% (770/935)</td>
</tr>
<tr>
<td>Do you eat chicken regularly?</td>
<td>88.7%</td>
</tr>
<tr>
<td>Did you eat chicken liver pate 5 days before feeling unwell</td>
<td>5.8% (47/814)</td>
</tr>
<tr>
<td>Did you handle raw chicken 5 days before feeling unwell</td>
<td>29.8% (247/830)</td>
</tr>
</tbody>
</table>
Table 5. Attributed source of isolates from cases who reported any animal exposure.

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Number</th>
<th>Attributed Source</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cattle</td>
</tr>
<tr>
<td>No Any animal exposure</td>
<td>890</td>
<td>0.11</td>
</tr>
<tr>
<td>Any animal exposure</td>
<td>110</td>
<td>0.16</td>
</tr>
</tbody>
</table>

The attributed STRUCTURE scores are presented horizontally and add up to a total of 1 or 100%. Higher score indicates higher likelihood of getting infected with *Campylobacter* from a specific reservoir.
3.4. The genomes and biology of *Campylobacter*

The availability of whole genome sequences of the isolates in this study enables a number of inferences about the biology and evolution of the two principal species to be made. Whilst the genomes are not closed sequences – they are typically spilt into a few tens of contigs – these will contain the vast majority of the genome of an isolate. In size they range from 1.6 - 1.9Mb with a majority around 1.7Mb. Some of this variation will be due to ‘losses’ in the gaps between contigs. This will also reduce the number of genes that can be detected in the genomes, as would erroneous assembly of the reads into contigs: for instance, homologous genes often do not assemble correctly (e.g. *flaA, flaB*). Each isolate comprises a set of ‘core’ genes which are common to all isolates in the species and encode housekeeping functions such as the machinery for DNA replication, transcription, translation, metabolism, cell structure and synthesis. An estimate of the number of genes in this core genome is illustrated in Figure 30 which uses as a reference strain *C. jejuni* NCTC11168 onto which the presence of genes in other isolates are mapped. The range in content is due to the greater or lesser similarity of each isolate to the NCTC11168 genome; however all isolates must harbour core genes. *C. jejuni* has a core genome of around 1450 genes. An estimate of the core genome which is common with *C. coli* would be around 1400, however there will be further genes in the *C. coli* core which are not present in *C. jejuni*. The rest of the genetic content of the isolates are ‘accessory’ genes and these will vary in presence or absence in different isolates and it is this variation that drives the total genome size variation that is seen in the isolates but is typically some 300 - 400 genes (Figure 31).

The biology of *Campylobacter* will be driven by a number of factors (Figure 32). In relation to the likelihood of initiation of human infection there must be both exposure to the organism and the strain must be capable of causing disease. Figure 11 illustrates that whilst there are some strains that are apparently host specific, many are generalists. The physiological state of an organism plays an important role in colonisation/pathogenicity and this is exemplified by the few secondary human campylobacteriosis cases that are detected compared to many other gastrointestinal bacterial pathogens (e.g. *E. coli* O157). For *Campylobacter*, an organism from a chicken can cause disease in humans but having passed through the human gut it is no longer pathogenic to other humans (Figure 32). The causes of *Campylobacter* pathogenesis are still poorly understood and it is difficult to differentiate pathogenic from non-pathogenic strains. Studies have shown that passage through the chicken reservoir promotes phase variation in contingency genes, and only these ‘successful’ variants colonise mice (Kim, Artymovich et al. 2012). This interplay between genotype and phenotype is not understood because all *Campylobacter* strains exhibit different levels of pathogenicity. Many of the chicken isolates tend to be pathogenic because the selective pressure in the chicken GI tract enriches low-abundance populations or enables the emergence of new successful subpopulations with large numbers of cells and new genotypes (Kim, Artymovich et al. 2012). The alteration of surface structure and/or regulation and expression of virulence factors such as motility or iron uptake results in enhanced colonisation and disease in mice and this is likely to be the case in human campylobacteriosis (Kim, Artymovich et al. 2012).
Our preliminary analyses to identify genes implicated in disease severity or in host specificity suggests that their effects are subtle and will require complex analysis. The identification of such genes will be of great value in tailoring molecular attribution analyses.

Figure 33 is a phylogenetic tree of all of the rST strain types detected in this study overlaid with the corresponding Clonal Complexes. Clonal Complexes are defined as STs which must have at least four of the seven loci in common with the central (ancestral) ST from which each complex is named. It is apparent that Clonal Complexes, which show groupings and are not random, do not correspond unambiguously with rST phylogeny indicating that the aggregation of similar STs into complexes is not evolutionarily robust. This report does not use Clonal Complex classifications as they are typically too coarse. There is a strong association between rST and traditional 7-locus ST, however there is not an increase in the stratification of isolates into strains with rST compared to ST, and so ST has been used in this report as the basis for strain classification.
Figure 30. Number of genes in isolates which are also in *Campylobacter jejuni* NCTC11168.
Figure 31. Pan-genome of *Campylobacter* and phylogenetic signal of gene presence.

Phylogenetic tree of *C. jejuni* (top branches of tree) and *C. coli* (bottom branch of tree) isolates mapping onto the presence (red) or absence (blue) of genes (open reading frames).
Survival and transmission within reservoirs (blue) and factors influencing likelihood of human infection (red). This is sometimes illustrated as “Source- Pathway- Receptor”.

Figure 32. Factors influencing the transmissibility of *Campylobacter* in reservoirs and the likelihood of successful infection of humans.
Figure 33. Phylogenetic tree based on rMLST.
Phylogenetic tree of all i-CaMPS3 rMLST types (based on allelic variation at 52 ribosomal loci) identified. Leftmost three images are *C. jejuni*, rightmost image is *C. coli*. (a) 7-locus MLST Sequence Types. Only those ST found in several rMLST types and are more abundant are indicated. (b) Clonal Complexes. All complexes are indicated with red symbol representing strains not in a recognised CC.
4. Conclusions and Implications for FSS strategy on *Campylobacter* reduction

The current study provides insights into the dynamic nature of *Campylobacter* and provides key baseline data on prevalence and strain types in the main food vehicles and animal reservoirs.

The study identifies continuing extensive population diversity of *Campylobacter* strains in farm animals, retail chicken and human isolates. The relative abundance of the strain types found in these reservoirs continues to be dynamic with even the relative abundance of the more common strains changing between the 2005-2007 study and this 2012-2015 study, indeed even over periods as short as one year. Notwithstanding this, the strain profiles in each reservoir species is characteristic of that particular host, and thus the basis of molecular attribution modelling continues to hold.

Host attribution modelling of putative sources of human infection identified that there continues to be broadly the same proportional attribution over all the study periods since 2005 with retail chicken being the largest contributor of *Campylobacter* infections, followed by cattle, sheep and other less common sources. From April 2012 to March 2015 source attribution modelling allocated clinical isolates to the following reservoirs: chicken 55-75%, sheep 10-22%, cattle 10 %, pigs 0-8% and wild birds 4-8%.

In previous studies (i-CaMPS, i-CaMPS-1, 2) the range of attribution for clinical isolates in Grampian varied from: 40% to 80% for chickens, 9-22% for cattle, 6% to 24% for sheep, 0% to 8% for pigs and 3% to 7% for wild birds.

The STs 21, 257, 48, 45, 50, 51 and 5136 were found to predominate in clinical isolates in this study. ST122 continued to increase in proportion since its first detection in 2012. Declining STs included ST21, ST53 and ST19.

In chicken samples there was a 10% decrease in the prevalence of *Campylobacter* compared to the i-CaMPS-1, -2 (2010-12) studies. ST50, one of the more common sequence types, has been increasing since 2011 (i-CaMPS-2). Similarly ST48 strains have increased proportionately since the previous studies (CAMPS, i-CaMPS-1,-2; 2005-12) to being the 2nd most common in the current study, and ST5136 has become the 3rd most common strain (found exclusively in chicken and turkey). The sequence types ST5, ST21, ST22, ST42, ST464 and ST1044 found to be in significant numbers in chicken during CaMPS, i-CaMPS-1,-2 (2005-2012), have decreased in abundance in the current study. The strains isolated from chicken tend to be very diverse which may be due to the susceptibility of chickens to a wide variety of strains and also to the extremely large size of poultry industry which effectively increases the size of this environmental niche.

The overall prevalence of *Campylobacter* in turkey in the current study was low compared to chicken, with STs 50, 5136, 45 and 51 commonly found in both hosts and STs 990, 583 and 137 exclusively found in turkey. The testing of strains from turkey in this study has identified another possible contributor source for human campylobacteriosis. It is probable that turkey does not play as major a role as chicken in human infections because there was no peak in the winter and because only 10% of overall poultry consumption is attributable to turkey.
The prevalence of *Campylobacter* in cattle has broadly remained the same since the i-CaMPS -1, -2 (2010-2012) studies, but has nearly doubled since the CaMPS (2005-2007) study. The most prevalent strains were STs 42, 827, 19, 61 and 21. The prevalence in sheep has increased by 15% since the i-CaMPS -1, -2 (2010-2012) study and has nearly doubled since the CaMPS study (2005-2007). The STs 19, 21, 61 and 827 that were found in significant numbers in cattle were also observed in sheep isolates. For these ruminant reservoirs - the second most important source of human infection - it is likely that human infection will be by direct contact with the animals or their faeces. Infections due to cattle or sheep associated strains were more common in people living in rural areas, particularly for the young, and this has been noted in our previous reports. Related to this, for cases reporting contact with animals, they were more likely to be infected with a non-chicken attributable stain of *Campylobacter*. Cases with a private water supply had an increased likelihood of infection by a strain attributable to a ruminant reservoir, however not at a significant level, whereas cases not on a private water supply did have an increased likelihood of infection with chicken attributable strain.

The *Campylobacter* prevalence in pigs in the current study was similar to the CaMPS study (2005-2007). The MLST types in pig isolates were not in the 30 most common strains found in other host reservoirs indicating that pigs were not a major cause of campylobacteriosis in Scotland.

Summer and autumn peaks for incidence of campylobacteriosis were observed, as seen in the previous studies. Although the cause of these are unknown, they appear not to be due to changes in the attributable sources of human infection.

This study has not captured any tangible impact from the initiatives in the UK *Campylobacter* control programme, however it is important to note the progress that has been in this area since the end of this study (FSA 2015a). The effects of forthcoming intervention strategies to reduce human campylobacteriosis originating from the poultry food chain should be observable by a decrease in human cases and confirmed by a subsequent decrease in the proportion of clinical isolates attributed to chicken/poultry, as has been observed in New Zealand (Sears, Baker et al. 2011).

This study is being followed up by a targeted project commissioned by FSS at the University of Aberdeen looking at the role of interventions on farms and at the abattoir (Coupar Angus) on *Campylobacter* contamination of retail chicken. This will have the potential to help us understand if broiler interventions in Scotland will have a positive impact on public health by reducing the number of clinical cases of IID attributable to *Campylobacter* from chicken sources.
5. Future work

The following are areas of future research that would build upon the existing knowledge from the work looking at the molecular attribution of Campylobacter:

- Utilising information from the whole genome sequencing carried out in this project, rather than just 7 locus MLST for source attribution and characterisation of strains.
- To use source attribution methods to assess the impact of any current industry interventions.
- The estimation of migration of strains between reservoirs is critical to our understanding of Campylobacter biology. This will influence the likelihood of being able to eradicate Campylobacter from the broiler farm. The commonality of many strains across host species shows the challenges that this will bring. Attribution using WGS may help elucidate this.
- Identifying the impact of interventions on farm and at abattoir (i-CaMPS-4 study) combined with an understanding of the role of strain types on these.
- Teasing out the interplay between Campylobacter reservoirs and human cases will lead to a fuller understanding of the importance of different sources for human infection: what is the relative exposure to each reservoir? This can be addressed by utilising the greater resolution of whole genome sequences. For example: WGS to fingerprint Campylobacter from farm to human cases may allow the tracking of actual routes.
- Is there a difference between C. jejuni and C. coli at the different points through the food chain? Should analyses consider these as separate species rather than combining them together through their commonality in human symptoms?
- Estimate the extent of strain diversification at a genome level (SNPs). Use the extent of polymorphisms in isolates from household cases or within broiler flocks to get base line information on the likelihood that groups of isolates are/not closely related. This would be of use in identifying whether isolates from human cases are part of a linked cluster (outbreak).
- What is the role of human deprivation on campylobacteriosis rates (ongoing FSS study FSS00016).
- Understanding the role of antimicrobial resistance mechanisms in Campylobacter. Previous reports suggests quinolone resistance has increased from 7% in 1995 to 37.5% in 2008 (Cody, Clarke et al. 2010). The main drivers of antimicrobial resistance are antimicrobial selection pressure and transmission. Farm to fork scenario will have a major role in the transmission of strains that are resistant which would have not only have an impact on the health of the individual but also on the economy. A recent report by researchers in China highlighted the importance of animal reservoirs (pigs and chicken) in transmission of the mcr-1 gene across the food chain to individuals infected by resistant bacterial strains (Liu, Wang et al. 2015). The levels of tetracycline resistance in Campylobacter are quite high. The widespread acquisition of antimicrobial resistance from isolates from retail poultry in the UK suggests horizontal gene transfer and the
proliferation of resistant lineage clusters which further indicates that conditions occur on poultry farms that favour resistant strains (Wimalarathna, Richardson et al. 2013). More research on this will shed light on the levels of antimicrobial resistance among Campylobacter isolates from retail poultry. This may clarify whether either drug resistant strains or indeed genes conferring resistance are impacting on humans.
6. Acknowledgements

We are very grateful to:

- The staff at Aberdeen Royal Infirmary NHS diagnostic microbiology laboratory for the provision of isolates and patient information.
- Grampian Health Board’s Health Protection Team for the circulation of the questionnaire and FSS operations team in Brechin, Turriff and Portlethen abattoirs for sample collection from different animals and for supplying beast information.
- Bird ringers in NE Scotland for providing wild bird faecal samples.
7. Recent outputs from this and related studies

7.1. Publications

7.2. Talks and Presentations

- “A longitudinal study of interventions and Campylobacter genotypes in British broiler farms” Presentation at CHRO, New Zealand, November 2015.
- Recommendations to Scottish Government on control of zoonoses: NS and KF contributed to discussions towards “Cooperative of Zoonoses Experience and Expertise (CoZEE) 2014 meeting and workshop: “The role of high-throughput sequencing in surveillance, diagnostics and tracking zoonotic infection”. Also two presentations on Campylobacter epidemiology and broilers.
- “Phenotypic viability of Campylobacter to relevant food chain production stresses”. Presentation at CampyUK, Liverpool, September 2014.
- “The origins of campylobacteriosis in NE Scotland; a comprehensive, co-ordinated Study.” Presentation at CampyUK, Liverpool, September 2014.
- “Phenotypic viability of Campylobacter to relevant food chain production stresses”. Presentation at Young Microbiologists’ Symposium, University of Dundee. June 2014.
- “Kitchen Killers” May Fest, public engagement with science event, Aberdeen, May 2014
- Norval Strachan, Ken Forbes and Minnie Ramjee spent a day in October 2013 filming with the BBC for a programme on ‘Campylobacter in chicken’ for the “Rip Off Britain” series, planned for broadcast in Jan 2014.
- Predictive microbiology and risk assessment course, Sao Paolo, Brazil, Dose Response, Nov 2013.
- International Association of Food Protection, Charlotte, USA on Source attribution and epidemiology of Campylobacter, Aug 2013.
- "Farm to Fork or There and Back Again” Hutton Institute Dundee Invited Seminar: April 2013
- NHS Grampian talks to Public Health on E. coli O157 and Campylobacter Research Talk, October and November 2012.

7.3. Posters

- Sparks N, Ruff L, Forbes Ken, Strachan NJC, Whyte F. Can on-farm biosecurity be used to control the prevalence of Campylobacter in the UK chicken flock? British Society of Animal Science, Nottingham, April 2014


7.4. Research Projects which have been assisted by i-CaMPS3

• Holden N, Brennan F, Forbes K, Strachan N (2013-16) “Pre-harvest contamination of fresh produce with VTEC” PhD Studentship, £60,000 from BBSRC EASTBIO

• Strachan N, Watts D, Forbes K, Perez-Reche F (2013-16) “Addressing food security by controlling the risk of food poisoning: an exemplar case study of listeriosis in the Scottish smoked salmon industry” PhD Studentship, £60,000 from University of Aberdeen Environment and Food Security Theme.


• Strachan N, Forbes KJ (2012) “Whole genome, molecular epidemiology of E.coli 0157 isolates from humans, food and the environment.” £11,000 from Food Standards Agency.


• Forbes KJ, Strachan N, Ogden ID, Sparks N (2012-14) “Integrating microbiology and modelling to determine the source of Campylobacter infection in the broiler house and develop interventions” £323,441 from BBSRC.
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Appendix Figure 1. Source attribution of Grampian clinical isolates in iCaMPS3 (2012-2015).

Source attribution of clinical isolates in iCaMPS3 using Source Reservoir Dataset. Error bars are 95% CI calculated by bootstrapping.
Confidence of correct attribution is assessed using self-attribution tests, that is the probability that the model will correctly assign an isolate to the source from which it was actually isolated. By chance for six hosts, there is a 17% likelihood that an isolate would be correctly attributed, and this threshold is indicated by the dashed line. Further details in i-CaMPS-2 final report. Error bars are 95% CI calculated by bootstrapping.