Public Health England

FSA Project FS102121

Year 2 Report

A microbiological survey of *Campylobacter* contamination in fresh whole UK-produced chilled chickens at retail sale

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Contents

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Abbreviations

BPW	Buffered Peptone Water
ºC	Degrees Celsius
GBRU	Gastrointestinal Bacteria Reference Unit
cfu	Colony forming units
CI	Confidence Interval
EQA	External Quality Assurance
FSA	Food Standards Agency
g	Gram
h	Hour(s)
PHE	Public Health England
IQA	Internal Quality Assurance
ISO	International Standard Organisation
l	Litre
LIMS	Laboratory Information Management System
mCCDA	modified Charcoal Cefoperazone Deoxycholate Agar
mg	Milligram
ml	Millilitre
MRD	Maximum Recovery Diluent
n	Number
OR	Odds Ratio
SOP	Standard Operating Procedure
spp.	Species
UK	United Kingdom
UKAS	United Kingdom Accreditation Service

Executive summary

Campylobacter spp. are the most common bacterial cause of foodborne illness in the UK, with chicken considered to be the most important vehicle for this organism. The joint FSA-industry target was set up to reduce the prevalence of the most contaminated chickens (those with > 1000 cfu per g chicken skin) to below 10 % at the end of the slaughter process, initially by the end of 2015 but has been rolled over to 2016.

A UK-wide survey was undertaken to determine the levels of *Campylobacter* spp. on whole fresh retail chickens and their packaging. The first survey year of data was collected by FSA Project FS241044 and this report represents results from sampling activity in the second survey year under FSA Project FS102121.

A total of 2998 samples of whole, UK-produced, fresh chicken was tested between July 2015 to March 2016 during this second survey year. The survey was suspended after March to allow for the trial of a modification to the analytical protocol.

The samples were evenly distributed throughout the UK (in proportion to the population size of each country) and testing was performed by six laboratory sites; five PHE and one laboratory in Northern Ireland (Agri-Food & Biosciences Institute, Belfast). Retailers were sampled evenly with their share of free-range and organic chickens taken into account.

For the method trial undertaken from April to July 2016, 416 chickens were examined to determine an alternative to using the standard 25 g of neck skin sample. Although these chickens were collected in accordance with the sampling protocol designed for the survey, they were used for experimental purposes and were therefore not included in the survey results. As such, the results for the work undertaken from April to July were recorded and analysed separately. No testing of outer packaging was performed on these samples.

Campylobacter enumeration on chicken samples was performed using the EN/TS/ISO 10272-2 standard enumeration method (applied with a detection limit of 10 cfu per g of skin or per outer packaging swab sample tested). During the first three sampling quarters (from July 2015 to the end of March 2016) two samples from each chicken pack were examined; one sample consisting of a 25 g chicken skin sample (mainly neck-skin), and another sample representing the outer packaging (examining a sponge swab that had been rubbed over the entire outer packaging of the chicken).

The proportion of *Campylobacter* spp. in fresh whole chicken at retail in the UK in the survey period from July 2015 to March 2016 was 61.3 %. Also in this time period, 11.4 % of samples had > 1000 cfu per g chicken skin. In 5.5 % of samples *Campylobacter* spp. were detected from the outer packaging swab ranging from 2.3 to 8.4 % between retailers. The *Campylobacter* spp. contamination found on the outer packaging was mostly at low levels, but levels of between 100 and 5740 campylobacter cfu per swab were detected in 1.2 % of samples.

There were significant differences in the proportion of highly contaminated chickens (ranging from 6.7 to 17.7 %) between retailers that could not be explained by differences in shelf-life remaining, chicken weights, sampling period or the type of rearing used. These comparisons were based on Q1 and Q2 alone due to concerns about lower neck-skin weight in samples in Q3. Comparing individual approval codes (signifying the slaughter house premises) also showed a significant difference in the proportion of chickens with >1000 cfu per g, ranging from 1.8 to 19.3 %, and it was noted that some retailers were predominantly supplied by specific approved premises.

A higher proportion of chickens had a high level of *Campylobacter* spp. during the first summer months quarter compared to during the subsequent months. The larger chickens, those with >1400 g in weight, showed a higher risk of being contaminated with >1000 cfu per g. There was no evidence of birds with access to range (e.g. free-range and organic birds) being more contaminated than birds reared under standard conditions but with much fewer free-range and organic birds tested there was limited precision in the comparison made.

For the majority of chicken skin samples (83.0 %) from which isolates were submitted for speciation, *C. jejuni* alone was identified. *Campylobacter coli* alone was identified in 13.5 % of samples. Both species were found in 3.4 % of samples. *Campylobacter coli* was more frequently isolated in the summer months and also more frequently isolated from birds with access to range. Where *C. jejuni* and/or *C. coli* speciation results were available from the chicken skin and the corresponding outer packing sample, the same species was detected in the large majority of samples.

The proportion of chicken on sale in the UK that are contaminated with a high level of campylobacters is considerable but chickens from some retailers are less contaminated suggesting it is possible to achieve better control of *Campylobacter* spp. in chicken. Data from this part year and the previous survey year has demonstrated a significant decline in the level of highly contaminated fresh whole UK retail chicken. The FSA has indicated that the retail proxy for the proportion of highly contaminated retail chickens should be less than 7 % and continued monitoring will be required to demonstrate a sustained decline.

From April to July 2016 the survey testing was ceased in order to undertake a method evaluation trial involving testing of carcass rinse, back-skin and neck-skin samples from the same carcass. This was done to address the concern regarding the differences in the weight of available neck-skin on chickens between retailers, which may hamper robust comparison of the results. The method evaluation trial included 416 chickens collected from different retailers in a similar manner as used in the survey.

The outcome of the method evaluation trial was to maintain testing of a neck-skin sample but with a reduction in the weight of sample tested to a maximum of 10 g (pure) neck-skin (allowing down to a 5 g sample where < 10 g neck-skin available) to ensure comparable samples from the large majority of chickens sampled.

1.0 Background

Campylobacter species, especially *Campylobacter jejuni,* are the main cause of human bacterial gastroenteritis in the developed world and it is estimated that there are in excess of half a million cases and 80,000 general practitioner consultations annually in the UK (Strachan *et al*. 2010, Tam *et al*. 2012). Source-attribution studies, outbreak investigations and case-control reports all incriminate chicken meat as the key food-borne vehicle for *Campylobacter* spp. infection, with cross contamination from poultry being identified as an important transmission route (Tam *et al*. 2009, Danis *et al*. 2009, Friedman *et al*. 2004; Mullner *et al*. 2009, Sheppard *et al*. 2009). Consumption of undercooked poultry or cross contamination from raw poultry meat is believed to be an important vehicle of infection (EFSA, 2009). Raw chicken meat is frequently contaminated with *Campylobacter* spp. and a decrease in the exposure levels from this source is likely to reduce the number of human cases of campylobacteriosis. The packaging of raw chicken has also been identified as a potential risk for infection. However, published data lack critical information on the levels detected on outer packaging and it is not known how levels on the outer packaging relate to levels on the chicken it contains (Jorgensen *et al*. 2002).

The UK Food Standards Agency (FSA) agreed with industry to reduce *Campylobacter* contamination in raw chicken and issued a target for this in order to measure the effectiveness of the FSA's Campylobacter Risk Management Programme (FSA 2009; 2010). The target was to reduce the percentage of chickens produced in UK poultry slaughterhouses (sampled at the post-chill stage) that are contaminated with >1,000 colony forming units (cfu) per g, from a 2008 baseline of 27 % to less than 10 % by December 2015. In theory, such a reduction would also be expected to be reflected in the levels found on chicken at retail sale, although fresh chicken sampled at retail may on average have lower levels of *Campylobacter* compared to those present immediately after slaughter, as *Campylobacter* spp. levels are known to reduce during the shelf-life of the chicken at retail-sale (Purnell *et al*. 2004).

The most important factor known to affect counts of *Campylobacter* spp. on a chicken carcass is the colonisation status of the chicken itself prior to slaughter (EFSA 2010a; Bull *et al*. 2006; Reich *et al*. 2008; Rosenquist *et al*. 2003). Studies have shown that when birds were not colonised at slaughter, *Campylobacter* spp. were either not detected or recorded as being present in very low numbers on carcasses (Allen *et al.* 2007). According to data from an EU survey, a colonised batch of chickens was 30 times more likely to result in a carcass that was contaminated with *Campylobacter* spp. than a non-colonised batch (EFSA 2010b). In the EU survey there was a very high proportion (70 %) of unexplained variance in *Campylobacter*-contamination results attributable to slaughterhouse-specific factors in colonised broiler batches for countries with a high prevalence, which included the UK. This is supported by other data, that identified different levels of *Campylobacter* contamination on carcasses despite carcasses originating from the same house and/or batch of birds sent for slaughter (Sampers *et al*. 2008; Figuerosa *et al*. 2009).

The prevalence of *Campylobacter* spp. in retail chicken, as determined by the standard ISO 10272-1 enrichment culture detection (presence/absence) method, has been associated with the time of year sampled (Meldrum 2005, CLASSP Project

Team 2010, Hutchison *et al*. 2006). However, the counts in post-chill chickens were not significantly associated with the month of sampling in the 2008 EU survey. The type of sample examined may also affect the counts obtained, but there is evidence that counts from carcass rinse and neck skin samples taken from the same chicken correlate well (Jorgensen *et al*. 2002).

Campylobacter spp. have been enumerated using conventional culture, ELISA, and methods based on DNA amplification (Jorgensen *et al*. 2002; Borck *et al*. 2002, Oyarzabal *et al*. 2005, Dufrenne *et al*. 2001, Hong *et al*. 2003; Wolffs *et al*. 2005; Fukushima *et al*. 2007). Accurate enumeration data are needed to support effective monitoring and risk assessment of *Campylobacter* spp. contamination in chicken meat and depend on the availability of reliable methods. *Campylobacter* spp. are fastidious bacteria with demanding growth requirements and this may challenge accurate and reliable detection and enumeration (Hutchison *et al*. 2006). While it is normally assumed that detection by enrichment culture is more sensitive than detection by direct plating, the EU survey reported instances where *Campylobacter* spp. were detected by enumeration but not by enrichment suggesting that the enrichment method yielded false negative results (EFSA 2010b). This has been reported elsewhere and may be associated with failure to grow *Campylobacter* sufficiently due to over-growth of other bacteria in the enrichment medium (Habib *et al*. 2008, Jasson *et al*. 2009). The EN/ISO/TS 10272-2 method recommended by the International Organisation for Standardisation provides a horizontal method for the enumeration of *Campylobacter* spp. involving direct plating onto modified charcoal cefoperazone desoxycholate agar (mCCDA) and incubation for 48 h at 41.5 °C (Anonymous, 2006). A collaborative study (Rosenquist *et al*. 2007) confirmed that direct plating on mCCDA is an acceptable protocol for the enumeration of thermotolerant *Campylobacter* spp. in chicken meat. The study, however, also found difficulties in detecting low numbers and variation between laboratories possibly due to difficulties in handling *Campylobacter* spp.. Direct spread plating on mCCDA has also been shown to be a reliable alternative to the most probable number method (Scherer *et al*. 2006).

In the EU survey about two-thirds of the *Campylobacter* spp. isolates from broiler carcasses were identified as *Campylobacter jejuni*, while one third was *C. coli* (EFSA 2010b). Speciation data is essential for meaningful epidemiological analysis and can allow accurate interpretation of antibiotic resistance data. With the introduction of molecular methods for determining species, these methods have been proven to be quick and reliable using species specific genes (Best *et al*. 2003, Melero *et al*. 2011).

The presence of *Campylobacter* spp. on the outer packaging of chicken packs has raised concern as consumers would not expect products to be contaminated on the outside and no specific instructions are provided with regard to the safe handling of such packaging before opening. Monitoring of quantitative data on the levels of *Campylobacter* spp. on outer packaging should continue until an acceptable level of risk is achieved.

In March 2012, the FSA put in place a new ongoing UK monitoring programme of chicken carcasses, sampled at post-chill. The FSA also completed a review, with stakeholders, of the joint campylobacter reduction target that was agreed in 2010, which has incorporated new data (FSA 2013). The FSA has developed a programme of initiatives from farm to fork to engage the whole of the food chain regarding the

control of *Campylobacter* spp. under the umbrella of the Acting on Campylobacter Together (ACT) campaign (FSA 2015a). In 2014-15, the FSA funded project FS241044 that looked to gather a year of data from whole raw chicken at retail sale. During that first survey year 4,011 samples of whole, UK-produced, fresh chicken from February 2014 to March 2015 were tested. The prevalence of *Campylobacter* spp. in the fresh chicken at retail in the UK found was 73.3 %. A significant proportion (19.4 %) of samples had > 1000 cfu per g chicken skin, and this ranged between retailers from 12.9 to 29.9 %. In 6.8 % of samples campylobacters were detected from the outer-packaging swab, this ranged between retailers from 3.1 to 12.5 %. The *Campylobacter* spp. contamination found on the outer packaging was mostly at low levels, but levels of between 100 and 4,500 *Campylobacter* spp. cfu per swab were detected in 1.6 % of samples. There were significant differences between retailers that could not be explained by differences in shelf-life remaining, chicken weights, time of year sampled or type of chicken rearing. Some approval codes (signifying the slaughter house premises) also showed a significant difference in the proportion of chickens with >1000 cfu per g, ranging from 9.4 to 29.7 %, and it was noted that some retailers were supplied by specific approved premises. A higher proportion of chickens had a high level of *Campylobacter* spp. during the summer compared to winter months. The larger chickens, those >1400 g in weight, showed a higher risk of being contaminated with >1000 cfu per g. There was no evidence of birds with access to range (e.g. free-range and organic birds) being more contaminated than birds reared under standard conditions but with much fewer free-range and organic birds tested no precise comparison could be made. For the majority of chicken skin samples (76.6 %) from which isolates were submitted for speciation, *C. jejuni* was identified. *C. coli* was identified in 13.9 % of samples. Both species were found in 4.2 % of samples. *Campylobacter coli* was more frequently isolated in the summer compared to winter and spring months and was more frequently isolated from birds with access to range. Where *Campylobacter* spp. was isolated from both the skin and the corresponding outer packing sample, the same species was detected in 93 % of these samples. As FS241044 identified that a significant proportion of chicken on sale in the UK remained contaminated and that therefore *Campylobacter* spp. in chicken continued to be important in terms of foodborne disease risk.

These findings led to the FSA, to continue the monitoring programme over three potential further years with project, FS102121:

- To determine the prevalence and levels of *Campylobacter* spp. contamination on fresh whole chilled chickens produced in the UK and sold at UK retail outlets by sampling up to a 36 month period, including annual breakpoints.
- To determine the prevalence and levels of *Campylobacter* spp. contamination found on the outside packaging of samples collected under Objective 1
- To identify *Campylobacter* spp. present and determine susceptibility of an agreed percentage of isolates to a defined range of antimicrobial agents.
- To collect information from each sample on a range of factors including bird weight, rearing method and type of packaging and determine any correlation with Campylobacter contamination.

In addition to the survey testing outlined above, a method evaluation trial was undertaken in the last part of project year 2. Concern had developed over the impact of the amount of breast skin used to supplement the sample weight when insufficient neck-skin was available. To address this concern, the survey protocol was stopped at the end of the third quarter to allow a revised work-plan for the final quarter to compare neck-skin, back-skin and whole carcass rinse samples in order to identify the most robust sample type to test in future years of the survey.

2.0 Methods

Sampling and testing procedures for the survey and the method evaluation work was agreed with the FSA (FSA 2015b; Appendix I).

The survey protocol used for the time period from July 2015 to March 2016 is briefly described below and is available from: http://www.food.gov.uk/sites/default/files/Campylobacter%20Retail%20Survey%20Year%202%20protocol%20%28final%29.pdf (FSA 2015b).

The method evaluation trial undertaken from April to July 2016 was carried out in two phases:

- In phase one, the standard 25 g neck-skin sample (supplemented with breast-skin if < 25 g neck-skin was available) was compared to a carcass-rinse (2.2.4) and a back-skin sample (2.2.3) from the same carcass.
- In phase two, testing of a 10 g neck-skin sample (with no breast-skin added even when < 10 g neck-skin was available) was compared to testing a carcass rinse (2.2.4) and a back-skin sample (2.2.3) from the same carcass (Appendix I).

Testing of outer packaging samples was suspended during the method evaluation trial.

2.1 Sampling method

Sampling was spread across the UK and designed to reflect population sizes. In contrast to survey year 1, a similar number of samples were obtained from each retailer. The numbers of free-range and organic chickens sampled within these were based on market share data from Kantar (FSA 2015b). Both samples for the survey and method evaluation trial were collected by trained individuals, who purchased samples from retail outlets and transported them to the appropriate testing laboratory according to the survey protocol. No samples from Scotland were included in the method evaluation trial. Samples On arrival at the laboratory, the air temperature of the cool boxes was taken using calibrated data loggers or temperature probes. Samples were documented using photographs and details were logged onto the laboratory information management system.

2.2 Microbiological methods

Six laboratories undertook the testing during the survey period Q1-3; five PHE Food, Water and Environmental Microbiology Service Laboratories and the Agri-Food & Biosciences Institute, Belfast. All laboratories enumerated campylobacters based on EN/ISO 10272-2 for the enumeration of *Campylobacter* spp. as detailed in the FSA survey protocol (FSA 2015) using mCCDA as the primary plating medium. All participating laboratories used the same method of achieving a microaerophilic atmosphere.

2.2.1 Outer packaging swab sample

Testing of outer packaging swab samples was performed during the three survey quarters as described previously using 10 ml of MRD and a dry SpongeSicle™ Swab. Enumeration of campylobacters was based on ISO 10272-2.

2.2.2 Neck/(Breast) Skin samples

These samples were prepared as described before using a 1:9 dilution of chicken neck-skin and buffered peptone water (BPW). Sample weights were 25 g skin (if < 25 g neck-skin available breast-skin was added) except for the chickens tested in the Phase Two part of the method trial where up to 10 g neck-skin only was used.

2.2.3 Back Skin samples

Back-skin samples were only tested during the method trial. A homogenate of a 1:10 dilution of back skin from chicken and BPW was prepared (ie. 25 g: 225 ml for Trial Phase One but 10 g: 90 ml in the Trial Phase Two). This was homogenised and plated in the same way as for neck-skin samples.

2.2.4 Chicken Carcass Rinse samples

Carcass rinse samples were only tested during the method trial. The entire chicken carcass was transferred to a sterile stomacher bag, with closures. A volume of 250 ml of BPW was added and the carcass was 'rinsed' for 1 minute, ensuring the media came into contact with every part of the chicken carcass. A volume of 3 ml was then transferred to a sterile universal as for the neck and back skin and was plated in the same manner.

2.3 Quality Assurance

During the previous FS241044 project a pilot study of 400 samples was initiated before commencing to establish and validate methods for sampling and enumerating *Campylobacter* spp. in samples from chickens and their packaging. The pilot provided the basis on which the current survey of whole UK-produced fresh retail chicken was developed.

All laboratories participate in recognised External Quality Assurance schemes, including the FSA funded scheme for enumeration of *Campylobacter* species, as well as operating comprehensive internal quality assurance schemes as part of the requirements of their accreditation to ISO 17025/2005 as assessed annually by the United Kingdom Accreditation Service (UKAS). All analyses were performed by trained and competent staff in a UKAS accredited laboratory operating an internal audit and review programme.

2.4 Statistical Analysis

Cross tabulations were analysed by the calculation of Clopper-Pearson exact 95 % confidence intervals for the proportion in each cfu per gram category. In addition, the Pearson chi square test of association has been used to test the null hypothesis of

no association between the measured variable and *Campylobacter* contamination. The expected counts in the individual cells of the table, together with the contribution to the overall chi square test statistics have been calculated to enable the identification of specific categories that determine the association.

Binary logistic regression analysis was used to assess whether any associations could be explained as a result of confounding by other important predictors of contamination. The outcome variable used was constructed around the FSA reduction target with the “positive” outcome defined as >1000 cfu per g, and a “negative” outcome being 1000 or fewer cfu per g.

For each predictor variable, the estimated odds ratios prior to and after adjustment for the confounding effects of the other important predictors were obtained from the logistic regression models. This enables an assessment of whether associations observed when a variable is assessed in isolation can be explained by confounding.

Factors examined were retailer, rearing regime, chicken weight, time of test in relation to shelf-life and sampling time period.

No post-hoc weighting for retailers market share was applied to any of the statistical analyses presented in this report

3.0 Results

Fresh raw whole UK produced chickens were collected from retail outlets across the UK between July 2015 and March 2016 (Figure 1). Retailers tend to use centralised distribution centres and therefore it is likely that similar chickens are sold in all their stores and because of this and considerations of transport times samples were mainly collected from sentinel urban areas.

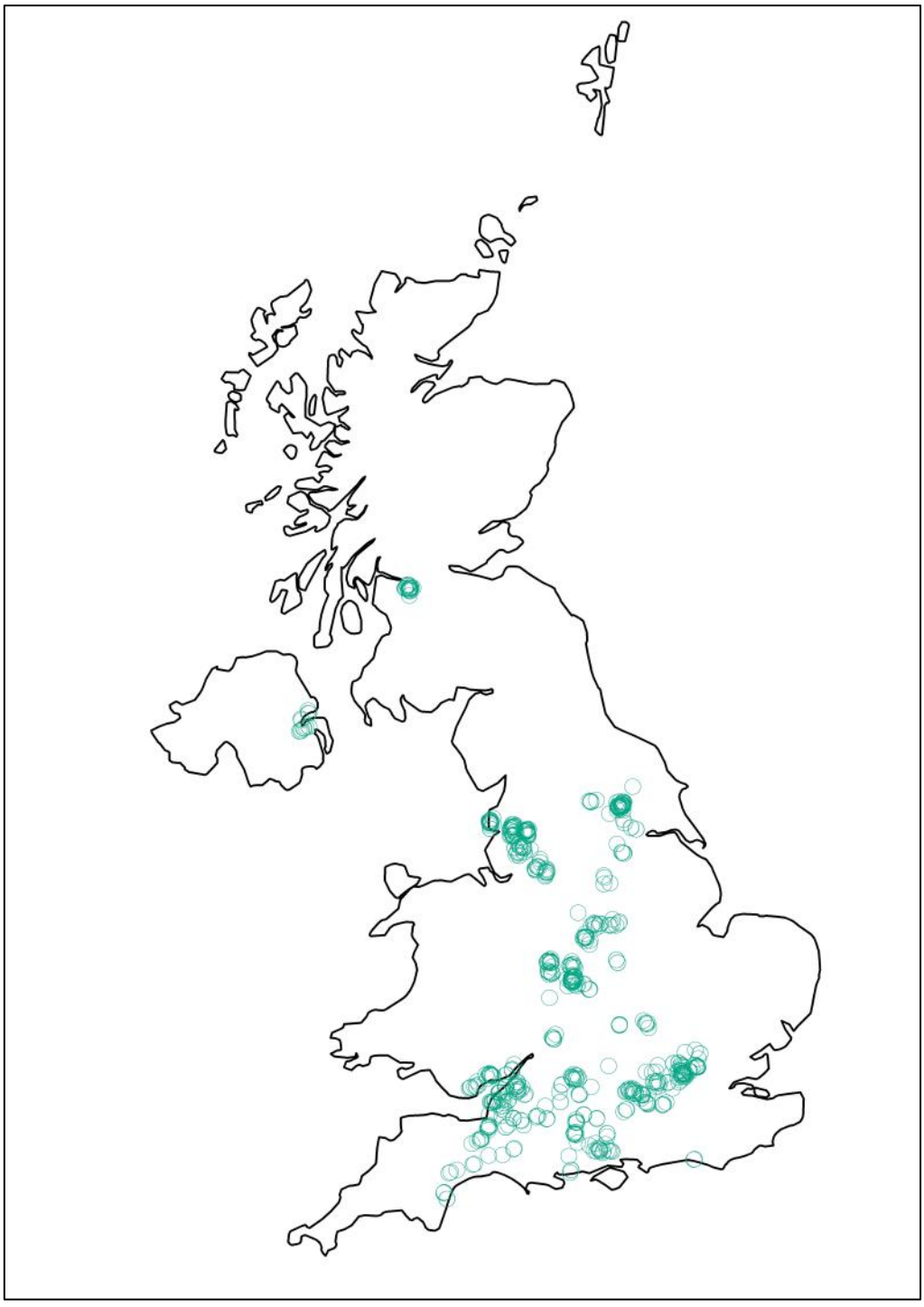

Figure 1. Geographical distribution of samples collected for the survey

3.1 Number of campylobacter in chicken skin and outer packaging samples from whole fresh UK produced chicken.

Based on all chickens examined during the survey period from July 2015 to March 2016, *Campylobacter* spp. were detected in the majority (61.3 %) of chicken skin samples and 11.4 % (95% CI = 10.3 to 12.6 %) of the skin samples (n = 2998 tested) had counts above 1000 cfu per g chicken skin. The highest count detected was 1,040,000 cfu of *Campylobacter* per g chicken skin. In outer packaging samples (n = 3002), *Campylobacter* spp. was detected in 5.5 % of samples.

3.1.1 *Campylobacter* spp. in chicken skin samples in relation to retailer.

The proportion of chickens with *Campylobacter* spp. levels at >1000 cfu per g ranged from 6.7 to 17.7 % across the retailer groups (Table 1). The proportion of chickens with *Campylobacter* spp. levels at >1000 cfu per g differed significantly between some of the retailers (Table 1). Possible confounding of these results was examined using logistic regression (see section 3.2).

Table 1. Number of Campylobacter spp. in retail chicken in relation to retailer

Retailer (n*)	**cfu of *Campylobacter* spp. per g chicken skin sample**							
	<10		**10-99**		**100-1000**		**> 1000**	
	n	**% (95 % CI)**	**n**	**% (95 % CI)**	**n**	**% (95 % CI)**	**n**	**% (95 % CI)**
Quarters 1 and 2								
Aldi (195)	59	30.3 (23.9 – 37.2)	51	26.2 (20.1 – 32.9)	69	35.4 (28.7 – 42.5)	16	8.2 (4.8 – 13.0)
Asda (199)	62	31.2 (24.8 – 38.1)	50	25.1 (19.3 – 31.7)	52	26.1 (20.2 – 32.8)	35	17.6 (12.6 – 23.6)
Co-op (197)	71	36.0 (29.3 – 43.2)	57	28.9 (22.7 – 35.8)	55	27.9 (21.8 – 34.7)	14	7.1 (3.9 – 11.6)
Lidl (195)	68	34.9 (28.2 – 42.0)	51	26.2 (20.1 – 32.9)	56	28.7 (22.5 – 35.6)	20	10.3 (6.4 – 15.4)
M&S (203)	63	31.0 (24.7 – 37.9)	42	20.7 (15.3 – 26.9)	64	31.5 (25.2 – 38.4)	34	16.7 (11.9 – 22.6)
Morrisons (201)	65	32.3 (25.9 – 39.3)	46	22.9 (17.3 – 29.3)	57	28.4 (22.2 – 35.1)	33	16.4 (11.6 – 22.2)
Sainsbury's (209)	56	26.8 (20.9 – 33.3)	54	25.8 (20.0 – 32.3)	62	29.7 (23.6 – 36.4)	37	17.7 (12.8 – 23.6)
Tesco (209)	64	30.6 (24.4 – 37.4)	58	27.8 (21.8 – 34.3)	66	31.6 (25.4 – 38.3)	29	10.0 (9.5 – 19.3)
Waitrose (194)	78	40.2 (33.2 – 47.7)	63	32.5 (25.9 – 39.6)	40	20.6 (15.2 – 27.0)	13	6.7 (3.6 – 11.2)
Others[#] (196)	71	36.2 (29.5 – 43.4)	33	16.8 (11.9 – 22.8)	63	32.1 (25.7 – 39.2)	29	14.8 (10.1 – 20.6)
Total (1998)	657	32.9 (30.8 – 35.0)	505	25.3 (23.4 – 27.2)	584	29.2 (27.2 – 31.3)	252	12.6 (11.2 – 14.1)
Total for Quarters 1 – 3								
Total (2998)	1160	38.7 (36.9 – 40.5)	710	23.7 (22.2 – 25.2)	786	26.2 (24.7 – 27.8)	342	11.4 (10.3 – 12.6)

*n = Number of samples

[#]Others included supermarkets with lower market shares and independents e.g. Iceland, convenience stores, butchers.

Direct retailer comparisons was omitted for Q3 as the variable trimming of neck skins across the industry (and the subsequent increasing amounts of breast skin used as a substitute for neck skin in the samples analysed), made comparisons increasingly less like for like.

3.1.2 Number of *Campylobacter* spp. in outer packaging samples in relation to retailer.

The prevalence and level of contamination found in the outer packaging samples was low. None of the retailers had a significantly different proportion of outer packaging samples positive for *Campylobacter* spp. compared to the overall average of 5.5 % based on survey quarters 1 -3 (Table 2).

Table 2. Number of *Campylobacter* spp. on outer packaging of retail chickens in relation to retailer (Quarters1-3)

Retailer (n*)	**cfu of *Campylobacter* spp. per outer packaging swab**							
	<10		**10-99**		**100-1000**		**>1000[a]**	
	n	**% (95 % CI)**	**n**	**% (95 % CI)**	**n**	**% (95 % CI)**	**n**	**% (95 % CI)**
Quarters 1 to 3								
Aldi (308)	296	96.1 (93.3 – 98.0)	11	3.6 (1.8 – 6.3)	0	0.0 (0.0 – 1.2)	1	0.3 (0.0 – 1.8)
Asda (309)	292	94.5 (91.3 – 96.8)	17	5.5 (3.2 – 8.7)	0	0.0 (0.0 – 1.2)	0	0.0 (0.0 – 1.2)
Co-op (282)	262	92.9 (89.3 – 95.6)	17	6.0 (3.6 – 9.5)	3	1.1 (0.2 – 3.1)	0	0.0 (0.0 – 1.3)
Lidl (287)	266	92.7 (8.9 – 9.5)	19	6.6 (4.0 – 10.1)	1	0.3 (0.0 – 1.9)	1	0.3 (0.0 – 1.9)
M&S (311)	304	97.7 (95.4 – 99.1)	4	1.3 (0.4 – 3.3)	3	1.0 (0.2 – 2.8)	0	0.0 (0.0 – 1.2)
Morrisons (301)	282	93.7 (90.3 – 96.2)	13	4.3 (2.3 – 7.3)	5	1.7 (0.5 – 3.8)	1	0.3 (0.0 – 1.8)
Sainsbury's (313)	298	95.2 (92.2 – 97.3)	10	3.2 (1.5 – 5.8)	5	1.6 (0.5 – 3.7)	0	0.0 (0.0 – 1.2)
Tesco (313)	300	95.8 (93.0 – 97.8)	10	3.2 (1.5 – 5.8)	3	1.0 (2.0 – 2.8)	0	0.0 (0.0 – 1.2)
Waitrose (294)	274	93.2 (89.7 – 95.8)	12	4.1 (2.1 – 7.0)	7	2.4 (1.0 – 4.8)	1	0.3 (0.0 – 1.9)
Others[#] (284)	262	92.3 (88.5 – 95.1)	18	6.3 (3.8 – 9.8)	3	1.1 (0.2 – 3.1)	1	0.4 (0.0 – 1.9)
Total (3002)	**2836**	94.5 (93.6 – 95.3)	**131**	4.4 (3.7 – 5.2)	**30**	1.0 (0.7 – 1.4)	**5**	0.2 (0.0 – 0.4)

*n = Number of samples

[#]Others included supermarkets with lower market shares (FSA 2015b) and independents e.g. Iceland, convenience stores, independents, butchers.

[a]The highest number of cfu of campylobacters recovered from an outer packaging sample was 5740.

3.1.3 Number of *Campylobacter* spp. in chicken skin samples in relation to chicken rearing regime

The rearing regime for chickens examined was recorded, and Table 3 summarises the levels of *Campylobacter* spp. detected in relation to whether the birds were reared without access to range (termed standard) or as free-range or as organic for Q1-3. Fewer samples from chickens reared using free range or organic production methods were examined to reflect their lower market share. This meant that, unless very large differences in contamination rates were present in these chicken types, it would not be possible to ascertain significant differences. Nevertheless, within this dataset, no significant differences in the proportion of highly contaminated chickens between the three types of chickens were found.

Table 3. Number of *Campylobacter* spp. in chicken in relation to bird rearing regime

Rearing regime (n*)	**cfu of *Campylobacter* spp. per g chicken skin sample**							
	<10		**10-99**		**100-1000**		**>1000**	
	n	**% (95 % CI)**	**n**	**% (95 % CI)**	**n**	**% (95 % CI)**	**n**	**% (95 % CI)**
Standard (2633)	1038	39.4 (37.5 – 41.3)	599	22.7 (21.2 – 24.4)	689	26.2 (24.5 – 27.9)	307	11.7 (10.5 – 12.9)
Free Range (328)	108	32.9 (27.9 – 38.3)	97	29.6 (24.7 – 34.8)	91	27.7 (23.0 – 32.9)	32	9.8 (6.8 – 13.5)
Organic (37)	14	37.8 (22.5 – 55.2)	14	37.8 (22.5 – 55.2)	6	16.2 (6.2 – 32.0)	3	8.1 (1.7 – 21.9)

*n = Number of samples

3.1.4 Number of *Campylobacter* spp. in chicken skin samples in relation to chicken processor approval number.

There were statistically significant differences in the distribution of contamination of chickens with *Campylobacter* spp. between the different processor approval numbers (i.e. slaughter house premises; Figure 2 and Table 4). The percentage of chickens with >1000 cfu per g ranged from 1.8 % for approval number 3005 to 19.3 % for approval number 4014.

Approval number 3005 and 9502 produced significantly fewer highly contaminated chickens compared to approval numbers 3007, 4014, 8005 and a group of other smaller production premises. Approval number 2037 also produced significantly fewer highly contaminated chickens compared to approval number 4014 and the group of other smaller production premises.

Table 4. Number of *Campylobacter* spp. in retail chicken in relation to processor

Processor Approval number (n*)	**cfu of *Campylobacter* spp. per g chicken skin sample**							
	<10		**10-99**		**100-1000**		**>1000**	
	n	**% (95 % CI)**	**n**	**% (95 % CI)**	**n**	**% (95 % CI)**	**n**	**% (95 % CI)**
Quarters 1 and 2								
1100 (116)	52	44.8 (35.9 – 54.3)	28	24.1 (16.7 – 33.0)	21	18.1 (11.6 – 26.3)	15	12.9 (7.4 – 20.4)
2037 (223)	75	33.6 (27.5 – 40.2)	67	30.0 (24.1 – 36.5)	62	27.8 (22.0 – 34.2)	19	8.5 (5.2 – 13.0)
3005 (55)	23	41.8 (28.7 – 55.9)	13	23.6 (13.2 – 37.0)	18	32.7 (20.7 – 46.7)	1	1.8 (0.0 – 9.7)
3007 (187)	45	24.1 (18.1 – 30.8)	54	28.9 (22.5 – 35.9)	61	32.6 (26.0 – 39.8)	27	14.4 (9.7 – 20.3)
3011 (81)	20	24.7 (15.8 – 35.5)	28	34.6 (24.3 – 46.0)	27	33.3 (23.2 – 44.7)	6	7.4 (2.8 – 15.4)
4014 (176)	45	25.6 (19.3 – 32.7)	35	19.9 (14.3 – 26.6)	62	35.2 (28.2 – 42.8)	34	19.3 (13.8 – 25.9)
5007 (36)	12	33.3 (18.6 – 51.0)	9	25.0 (12.1 – 42.2)	10	27.8 (14.2 – 45.2)	5	13.9 (4.7 – 29.5)
5011 (325)	122	37.5 (32.3 – 43.1)	68	20.9 (16.6 – 25.8)	100	30.8 (25.8 – 36.1)	35	10.8 (7.6 – 14.7)
5464 (28)	10	35.7 (18.6 – 55.9)	6	21.4 (8.3 – 41.0)	8	28.6 (13.2 – 48.7)	4	14.3 (4.0 – 32.7)
8005 (319)	90	28.2 (23.3 – 33.5)	79	24.8 (20.1 – 29.9)	98	30.7 (25.7 – 36.1)	52	16.3 (12.4 – 20.8)
9502 (235)	106	45.1 (38.6 – 51.7)	77	32.8 (26.8 – 39.2)	40	17.0 (12.4 – 22.4)	12	5.1 (2.7 – 8.7)
Other code[#] (168)	41	24.4 (18.1 – 31.6)	33	19.6 (13.9 – 26.5)	62	36.9 (29.6 – 44.7)	32	19.0 (13.4 – 25.8)
Not Available[§] (49)	16	32.7 (19.9 – 47.5)	8	16.3 (7.3 – 29.7)	15	30.6 (18.3 – 45.4)	10	20.4 (10.2 – 34.3)
Quarters 1 – 3								
Total (2998)	1160	38.7 (36.9 – 40.5)	710	23.7 (22.2 – 25.2)	786	26.2 (24.7 – 27.8)	342	11.4 (10.3 – 12.6)

*n = Number of samples

[#]Samples listed within the 'Other code' category had < 50 chickens from the processor sampled within the study. A list of approved premises codes can be found on the FSA website http://www.food.gov.uk/enforcement/sectorrules/meatplantsprems/meatpremlicence

[§]Shop was unable to provide processor Approval number

3.1.5 Number of *Campylobacter* spp. in chicken skin samples in relation to quarter tested.

Significant variation was detected in the levels of the bacterium present for the different sampling quarters. A higher proportion of chickens had a high level contamination of *Campylobacter* spp. during the first quarter dominated by summer months compared to the subsequent Q3 winter months (Table 5).

Table 5. Number of *Campylobacter* spp. in retail chicken in relation to season

Quarter (n*)	**cfu of *Campylobacter* spp. per g chicken skin sample**							
	<10		**10-99**		**100-1000**		**>1000**	
	n	**% (95 % CI)**	**n**	**% (95 % CI)**	**n**	**% (95 % CI)**	**n**	**% (95 % CI)**
1 July/Aug/Sep 2015 (1032)	257	24.9 (22.3-27.7)	284	27.5 (24.8-30.4)	343	33.2 (30.4-36.2)	148	14.3 (12.3-16.6)
2 Oct/Nov/Dec 2015 (966)	400	41.4 (38.3-44.6)	221	22.9 (20.3-25.7)	241	24.9 (22.2-27.8)	104	10.8 (8.9-12.9)
3 Jan/Feb/Mar 2016 (1000)	503	50.3 (47.2-53.4)	205	20.5 (18.0-23.1)	202	20.2 (17.8-22.8)	90	9.0 (7.3-10.9)

*n = Number of samples

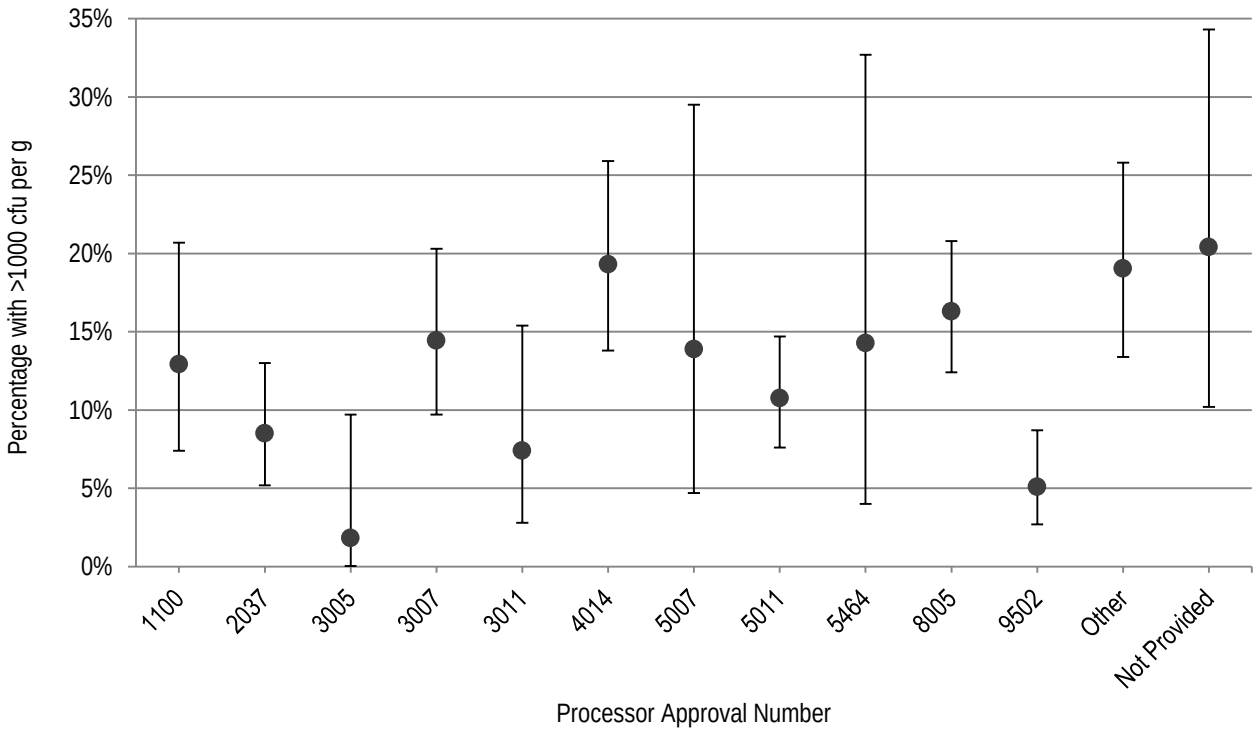

Figure 2. The percentage of chickens with >1000 cfu of campylobacters per g chicken skin in relation to processor approval number. Samples listed within the 'Other' code category had < 50 chickens from the processor sampled within the study. A list of approved premises codes can be found on the FSA website http://www.food.gov.uk/enforcement/sectorrules/meatplantsprems/meatpremlicence

3.1.6 Number of *Campylobacter* spp. in chicken skin samples in relation to chicken weight

Chickens were assigned into three weight categories defined by arbitrary weight ranges based on reviewing weights of chickens listed as 'small' or 'medium' or 'large' (Table 6). Assignment of a size category to the chicken purchased allowed the separation of the data. This enabled analysis to determine whether size, which may be linked to the age of the chicken at slaughter, is associated with the level of *Campylobacter* spp. present. Using these categories, medium and large birds had a statistically significantly higher number of samples with >1000 cfu of *Campylobacter* spp. per g (Table 6).

Table 6. Number of *Campylobacter* spp. in retail chicken in relation to chicken weight

Chicken weight (n*)	**cfu of *Campylobacter* spp. per g chicken skin sample**							
	<10		**10-99**		**100-1000**		**>1000**	
	n	**% (95 % CI)**	n	**% (95 % CI)**	n	**% (95 % CI)**	n	**% (95 % CI)**
Small <1400 g (982)	445	45.3 (42.2 – 48.5)	232	23.6 (21.0 – 26.4)	219	22.3 (19.7 – 25.3)	86	8.8 (7.1 – 10.7)
Medium 1400-1750 g (1389)	529	38.1 (35.5 – 40.7)	330	23.8 (21.5 – 26.1)	372	26.8 (24.5 – 29.2)	158	11.4 (9.8 – 13.2)
Large >1750 g (614)	179	29.2 (25.6 – 32.9)	146	23.8 (20.5 – 27.3)	193	31.4 (27.8 – 35.3)	96	15.6 (12.9 – 18.8)

*n = Number of samples; no weight data was available for 13 chickens.

3.1.7 Number of *Campylobacter* spp. in chicken skin samples in relation to days of shelf-life remaining

Chickens were tested with up to nine days of remaining shelf-life (Table 7). At testing, the most frequent number of days of shelf-life remaining was 4-5 days. There was no association detected between high level contamination and the length of shelf-life remaining in days, i.e. no association with those birds that are closer to their production date.

Table 7. Number of *Campylobacter* spp. in retail chicken in relation to days of remaining shelf-life (Quarters 1-3)

Remaining shelf-life in days (n*)	**cfu of *Campylobacter* spp. per g chicken skin sample**							
	<10		**10-99**		**100-1000**		**>1000**	
	n	**% (95 % CI)**	n	**% (95 % CI)**	n	**% (95 % CI)**	n	**% (95 % CI)**
0-1 (79)	35	44.3 (33.1 – 55.9)	21	26.6 (17.3 – 37.7)	18	22.8 (14.1 – 33.6)	5	6.3 (2.1 – 14.2)
2-3 (747)	303	40.6 (37.0 – 44.2)	176	23.6 (20.6 – 26.8)	188	25.2 (22.1 – 28.4)	80	10.7 (8.6 – 13.2)
4-5 (1362)	514	37.7 (35.2 – 40.4)	312	22.9 (20.7 – 25.2)	370	27.2 (24.8 – 29.6)	166	12.2 (10.5 – 14.0)
6-7 (725)	277	38.2 (34.7 – 41.9)	184	25.4 (22.2 – 28.7)	182	25.1 (22.0 – 28.4)	82	11.3 (9.1 – 13.8)
8-9 (78)	29	37.2 (26.5 – 48.9)	17	21.8 (13.2 – 32.6)	25	32.1 (21.9 – 43.6)	7	9.0 (3.7 – 17.6)
Not available (7)	2		0		3		2	

*n = Number of samples

3.1.8 Other factors

Whilst the protocol stipulated to test a 25 g neck-skin sample not all chickens had sufficient neck-skin available to allow this weight to be tested. Where less than 25 g neck skin was available in Q1-3, the remaining weight was made up to 25 g using breast skin from the same carcass. The average grams of neck-skin in samples differed between retailers, with the “Others” and Sainsbury’s having the highest average amount in samples (Figure 3). It is possible that the level of cfu of *Campylobacter* spp. per g skin may be affected by the total weight of neck-skin used, however the data from the previous survey year (PHE 2015) indicated that while the proportion of neck-skin influenced the contamination rate, it did not confound the association between retailer and the proportion of highly contaminated chickens found.

Some retailers consistently sold chickens packed using a modified atmosphere packaging (MAP) whilst the large majority of chickens obtained from butchers were not MAP packed. MAP packing was therefore highly correlated with retailer type. For a proportion of chickens it proved difficult to ascertain from the packaging whether the chicken was in fact packed using MAP or not, thus making detailed analysis problematic. *Campylobacter* spp. are microaerophilic bacterial genus and do not tolerate atmospheric oxygen levels as effectively as aerobic organisms and it is possible that higher levels of oxygen could decrease survival (Blankenship & Craven, 1982; Grigoriadis *et al*., 1997).

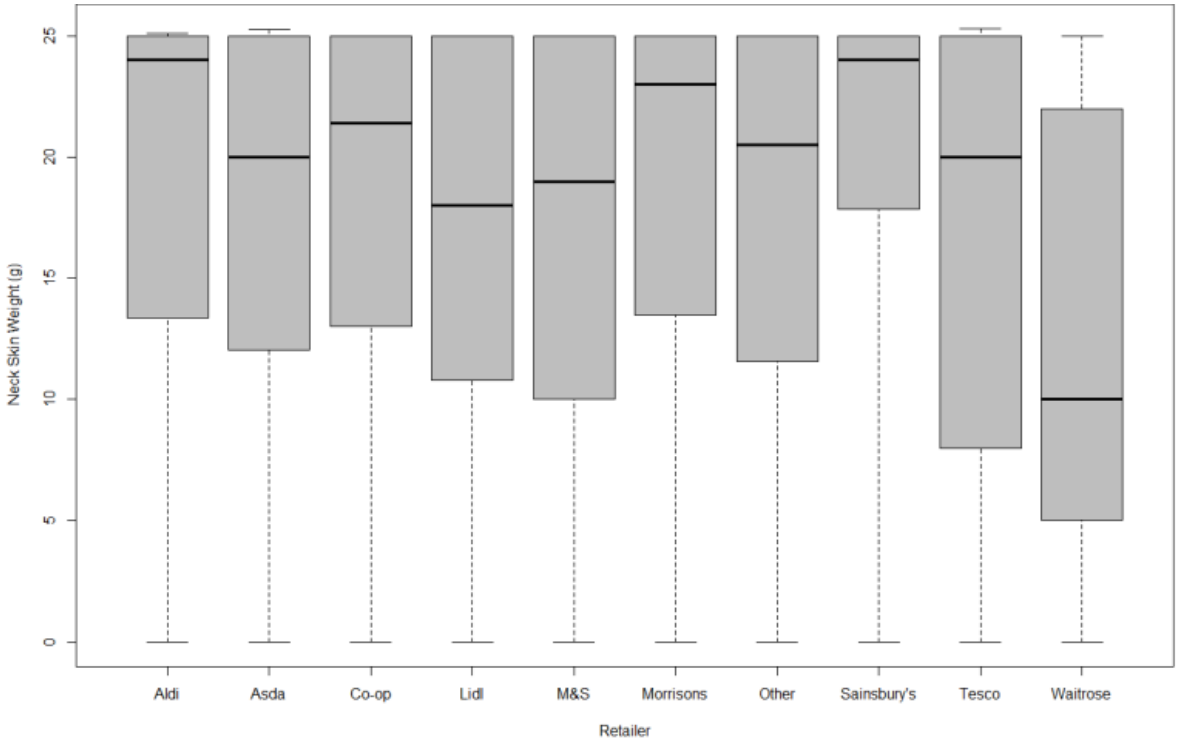

Figure 3. Chicken neck-skin weight in samples in relation to retailer (Quarters 1-2)

3.2 Logistic regression

Analysis of the cfu of *Campylobacter* per g of chicken skin did not detect noticeable confounding factors and the multivariable logistic regression model provided very similar estimates of odds ratios to those obtained when each variable was considered in isolation in the single variable logistic regression analysis (Table 8).

This indicated that the variation in the percentage contamination in chickens from the different retailers could not be explained by chicken type, quarter of sampling, days of shelf-life remaining or chicken weight, and as such is likely to represent genuine variation between the retailers. The group of smaller independent retail outlets (i.e. the group termed “Others”), Sainsbury’s, Asda, M&S and Morrisons were all significantly different to the “reference” Co-op (selected as reference as set as reference in the previous survey year). It was decided that the analysis should be focused around differences between retailers, in line with the interim publications of the accumulated study data produced by the FSA (FSA 2016).

Due to the relationship between retailers and processors it was not possible to separate any individual association they may have with high level *Campylobacter* spp. contamination. It is likely that the processor has a bearing on contamination rate and this will be manifested as variations in the contamination rate between retailers. As retailers may source chickens from multiple processors, it would be difficult for consumers to make informed choices on the basis of information about the processor and hence processor was not included in the logistic regression model.

Table 8. Estimated odds ratios from single variable and multivariable logistic regression models of *Campylobacter* spp. contamination levels >1000 cfu per g chicken skin

Variable	Single variable analysis		Multivariable analysis	
	OR (95% CI)	p-value	OR (95% CI)	p-value
Retailer		<0.001		<0.001
Co-op	Reference		Reference	
Aldi	1.17 (0.55 to 2.46)		1.22 (0.57 to 2.62)	
Asda	2.79 (1.45 to 5.37)		3.36 (1.72 to 6.54)	
Lidl	1.49 (0.73 to 3.05)		1.62 (0.79 to 3.34)	
M&S	2.63 (1.36 to 5.07)		3.24 (1.65 to 6.36)	
Morrisons	2.57 (1.33 to 4.96)		2.64 (1.36 to 5.21)	
Sainsbury's	2.81 (1.47 to 5.38)		3.28 (1.67 to 6.43)	
Tesco	1.46 (0.72 to 2.96)		1.66 (0.80 to 3.41)	
Waitrose	0.94 (0.43 to 2.05)		1.20 (0.54 to 2.61)	
Other	2.27 (1.16 to 4.44)		2.72 (1.35 to 5.49)	
Chicken type		0.11		0.11
Standard	Reference		Reference	
Free Range	0.64 (0.40 to 1.04)		0.67 (0.41 to 1.10)	
Organic	0.45 (0.11 to 1.91)		0.35 (0.08 to 1.50)	
Quarter[a]		0.02		0.01
Quarter 1	1.39 (1.06 to 1.81)		1.42 (1.08 to 1.87)	
Quarter 2	Reference		Reference	
Remaining shelf-life		0.3		0.11
Per additional day	1.05 (0.96 to 1.14)		1.08 (0.98 to 1.19)	
Weight		0.006		0.002
Small <1400 g	Reference		Reference	
Medium 1400-1750 g	1.34 (0.97 to 1.84)		1.51 (1.08 to 2.13)	
Large >1750 g	1.81 (1.26 to 2.61)		1.98 (1.35 to 2.90)	

[a]For the purposes of this report, Q1 was defined as July, August and September 2015; Q2 as October, November and December 2015.

3.3 Campylobacter species isolated from skin and outer packaging samples of fresh whole UK produced chicken at retail

Isolates from a total of 1685 chicken neck skin samples were subjected to *C. jejuni/C. coli* speciation testing. *C. jejuni* alone was found in 83.0 %, *C. coli* alone in 13.5 %, both species in 3.4 % of samples (Table 9). For 12 samples neither *C. coli* nor *C. jejuni* were detected and no speciation test was available for 153 samples due to loss of isolate viability.

Table 9. *Campylobacter* spp. isolates from retail chicken skin samples

Species detected	No. of samples	% of samples[a]
***C. jejuni* (only)**	1399	83.0
***C. coli* (only)**	228	13.5
C. jejuni* and *C. coli	58	3.4

[a]Samples (1685) from where an isolate (or isolates) was identified as either *C. jejuni* or *C. coli* or both of these species (reflecting a mixed isolation).

C. coli alone was significantly more frequently isolated during Q1 (18 %), covering the Summer months, compared the rest of the year (10 %) ($p < 0.001$; Fisher's exact test) (Table 10). Conversely, the proportion of samples from which *C. jejuni* was isolated was lower in Q1 (77 %) compared to the remainder of the year (87 %).

Table 10. *Campylobacter jejuni* and *C. coli* isolates from retail chicken skin samples

Species detected	Quarter[a] % of samples with species (no. of samples)		
	Q1 (n = 675)	**Q2** (n = 532)	**Q3** (n = 478)
***C. jejuni* only**	77 (520)	85 (453)	89 (426)
***C. coli* only**	18 (123)	12 (64)	9 (41)
Mixed *C. jejuni* & *C. coli*	5 (32)	3 (15)	2 (11)

[a]For the purposes of this report, Q1 was defined as July, August and September 2015; Q2 as October, November and December 2015; Q3 as January, February and March 2016

The proportion of *C. coli* isolated from chickens reared as free-range or organic was significantly higher than from chickens reared without access to range (termed standard rearing; $p < 0.001$ and < 0.05 for free-range or organic, respectively; Fisher's exact). However, further data would be required to ascertain this observation as only a small number of organic birds was tested. Nonetheless, the probability of observing 6 of the 24 tested as positive for *C. coli* is very small if the true proportion of positives is 0.136 ($p = 0.01$ exact binomial test) (Table 11).

Table 11. *Campylobacter jejuni* and *C. coli* isolates from retail chicken skin samples in relation bird rearing regime

Species detected	Chicken rearing method % of samples with *Campylobacter* species (no. of samples)		
	Standard rearing (no access to range) (n = 1457)	**Free range** (n = 205)	**Organic** (n = 23)
***C. jejuni* only**	85 (1245)	67 (138)	70 (16)
***C. coli* only**	11 (167)	27 (55)	26 (6)
C. jejuni* and *C. coli	3 (45)	6 (12)	4 (1)

For some processor approval numbers, a slightly higher proportion of *C. coli* appeared to be isolated compared to the average for all approval numbers and vice-versa for other processors a higher proportion of *C. jejuni* was found (Table 12).

Table 12. *Campylobacter jejuni* and *C. coli* isolates from retail chicken in relation to processor

Quarters 1 – 2						
Processor Approval number	***C. jejuni* only**		***C. coli* only**		***C. jejuni* & *C. coli***	
	%	**No. of samples**	**%**	**No. of samples**	**%**	**No. of samples**
1100	87.1	54	9.7	6	3.2	2
2037	87.8	115	7.6	10	4.6	6
3005	88.5	23	3.8	1	7.7	2
3007	90.7	117	7.0	9	2.3	3
3011	83.9	47	12.5	7	3.6	2
4014	70.6	84	23.5	28	5.9	7
5007	70.8	17	16.7	4	12.5	3
5011	83.5	152	14.8	27	1.6	3
5464	40.0	6	33.3	5	26.7	4
8005	81.8	166	15.3	31	3.0	6
9502	72.9	86	24.6	29	2.5	3
Other code[#]	74.6	85	21.9	25	3.5	4
Not Available[§]	75.0	21	17.9	5	7.1	2
Quarters 1 – 3						
Total	83.0	1399	13.5	228	3.4	58

[#]Samples listed within the 'Other code' category had < 50 chickens from the processor sampled within the study. A list of approved premises codes can be found on the FSA website http://www.food.gov.uk/enforcement/sectorrules/meatplantsprems/meatpremlicence

[§]Shop was unable to provide processor Approval number.

Isolates from a total of 146 outer packaging samples were subjected to *C. jejuni/C. coli* speciation testing. *C. jejuni* alone was found in 83.1 %, *C. coli* alone in 13.7 %, both species in 2.1 % of samples (Table 13). For 2 samples neither *C. coli* nor *C. jejuni* were detected and no speciation test was available for 18 samples due to loss of isolate viability.

Table 13. *Campylobacter jejuni* and *C. coli* isolates from outer packaging of retail chicken

Species detected	**No. of samples**	**% of total samples speciated** (n = 146)
***C. jejuni* only**	123	83.1
***C. coli* only**	20	13.7
C. jejuni* and *C. coli	3	2.1

Comparison of isolates from 133 samples where *C. jejuni/ C. coli* speciation data was available from both the outer packaging sample and the corresponding skin sample showed that the same species was detected in the large majority of samples (Table 14). However, on ten occasions (7.5 %) a different *Campylobacter* species was detected in the two samples that had been derived from the same chicken pack.

Table 14. Number of chickens with *Campylobacter jejuni* and/or *C. coli* species in outer packaging and corresponding chicken skin sample

***Campylobacter* species detected in skin sample**	***Campylobacter* species detected in outer packaging swab sample**		
	***C. jejuni* only**	***C. coli* only**	***C. jejuni* and *C. coli***
***C. jejuni* only**	110	7	1
***C. coli* only**	3	9	0
C. jejuni* and *C. coli	2	0	1

3.4 Method evaluation trial

The laboratory protocol for projects FS241044 and FS102121 were based on measuring the amount of Campylobacter on 25 g of chicken neck skin (generally the most contaminated part of the bird). However, analysis of data collected in Quarters 1 to 3 (Q1-3) by laboratories testing whole raw chickens at retail identified that a lower proportion of chickens examined in 2016 had greater than 10 g of neck skin available for testing compared to 2015. Preliminary analysis showed that *Campylobacter* levels may be lower for 25 g samples when a smaller amount of neck-skin is present in samples (where if < 25 g neck-skin was available, breast-skin was used to achieve a total of 25 g; PHE 2015). The increasing use of breast skin in the sample over time, and differences in the amount of breast skin that had to be included between retailers, may have introduced a greater variation between the samples in the survey making equitable retailer to retailer comparisons and accurate comparisons with previous quarterly results difficult.

On identification of this problem, the study protocol was stopped at the end of Year 2, Q3 to allow a revised workplan to evaluate samples other than neck-skin from the chicken in terms of suitability (i.e. a sample that will allow robust comparisons in the long term), feasibility/practicability and impact on results. Two alternative samples were suggested: a carcass rinse sample and a back-skin sample. The carcass rinse sample has been widely used e.g. in the Coordinated Local Authority Sentinel Surveillance of Pathogens (CLASSP) study that was undertaken by local authorities and the then Health Protection Agency (HPA) in England between the 1st November 2004 and the 31st October 2007 examining > 2000 whole raw chickens, whilst literature reviews identified no evidence of another survey utilising back-skin as a sample. The latter may reflect a concern that a back-skin sample may be subject to highly variable faecal contamination associated with uneven back skin contamination events, especially at evisceration. It was recognised that whilst a new sample would result in a different baseline and potentially a different measure unit, it may be useful to have data on how the standard neck/breast-skin sample relates to a new sample type. To address this, each individual chicken was examined by undertaking *Campylobacter* spp. enumeration of a neck-skin sample, a back-skin sample and a carcass rinse sample.

To evaluate an alternative sampling methodology approach for the accurate assessment of *Campylobacter* spp. contamination on chicken carcasses that fulfils several key criteria:

- Feasibility/ practicability in the testing laboratory (consistent testing)
- Ensures the highest level of robustness of data and comparability of enumeration results between retailers
- Reliable and robust in the long-term (not liable to become obsolete)

Following the suspension of the survey a total of 416 fresh raw whole UK produced chickens was collected across retail outlets between April and July 2016 (Table 15) to be used in the method evaluation trial. *Campylobacter* were detected in 208 neck-skin (50 %), 218 back-skin (52 %) and 280 (67 %) carcass-rinse samples of the 416 chickens tested.

Table 15. Number of samples with levels of *Campylobacter* spp. found and neck-skin weights available for method evaluation trial

	cfu of Campylobacter spp. g^{-1} neck-skin			% of samples within neck-skin weight category (no. of samples)	
	< 10	10-1000	> 1000		
	n	n	n	< 10 g	< 5 g
Total (416)	208	187	21	30 (124)	6 (23)

Testing carcass rinse samples resulted in significantly more chickens testing Campylobacter positive than testing neck-skin or back-skin samples (McNemar; $P < 0.001$; Table 16). *Campylobacter* spp. were detected in 185 chickens in all 3 sample types and not detected in any of the samples for 128 chickens while for 288 (69 %) chickens campylobacters were detected in at least one sample.

Detection agreement between the three samples was assessed statistically by calculating Fleiss' kappa (using http://dfreelon.org/utils/recalfront/recal3/) with data categorised as detected or not detected (Table 16). According to this test there was an overall good to fair agreement between the test results obtained using the three sample types. The strongest detection agreement was between neck and back skin samples, while a slightly lower level of agreement was found between back-skin or neck-skin and carcass rinse samples.

The level of *Campylobacter* cfu in the three sample types were compared using $\log_{10}$ cfu of *Campylobacter* per g skin and per ml rinse. While *Campylobacter* in carcass-rinse samples were measured using a different unit it may be reasonable to make comparisons considering a total skin weight could approximate 250 g and thus for the chicken as a whole recovery from 1 g may relate to 1 ml of rinse as the total rinse volume was 250 ml. Assigning counts to categories as either < 1.15, 1.15 – 3 or > 3 and then calculating agreement showed good overall agreement between the three sample types.

Table 16. Detection of *Campylobacter* spp. in retail chicken using different sample types

	Sample type	Number of chickens where *Campylobacter* were DETECTED in sample indicated		
		Neck-skin	Back-skin	Carcass rinse
Number of chickens where *Campylobacter* were NOT detected	**Neck-skin**	-	32	77
	Back-skin	22	-	66
	Carcass-rinse	5	4	-

There was a reasonable agreement between counts for back and neck-skin samples (Table 17; Figure 4) and a McNemar test did not detect any significant difference in

the proportion of chicken with > 1000 cfu of *Campylobacter* spp. per g between neck-skin and back-skin samples.

Table 17. *Campylobacter* spp. levels in neck-skin and back-skin samples from 416 retail chickens

	Log_{10} *Campylobacter* per g chicken	**Number of chickens with level of *Campylobacter* in neck-skin sample**		
		< 1.15	**1.15 – 3**	**> 3**
Number of chickens with level of *Campylobacter* in back-skin sample	**< 1.15**	195	21	2
	1.15 – 3	34	137	15
	> 3	0	8	4

There was a reasonable agreement between counts from neck-skin and carcass-rinse samples (Table 18; Figure 5) and a McNemar test did not detect any significant difference in the proportion of chicken with > 1000 cfu of *Campylobacter* spp. per g or ml between neck-skin and carcass-rinse samples.

Table 18. *Campylobacter* spp. levels in neck-skin and carcass-rinse samples from 416 retail chickens

	Log_{10} *Campylobacter* per g or ml	**Number of chickens with level of *Campylobacter* in neck-skin sample**		
		< 1.15	**1.15 – 3**	**> 3**
Number of chickens with level of *Campylobacter* in carcass rinse sample	**< 1.15**	188	18	0
	1.15 – 3	41	134	13
	> 3	0	14	8

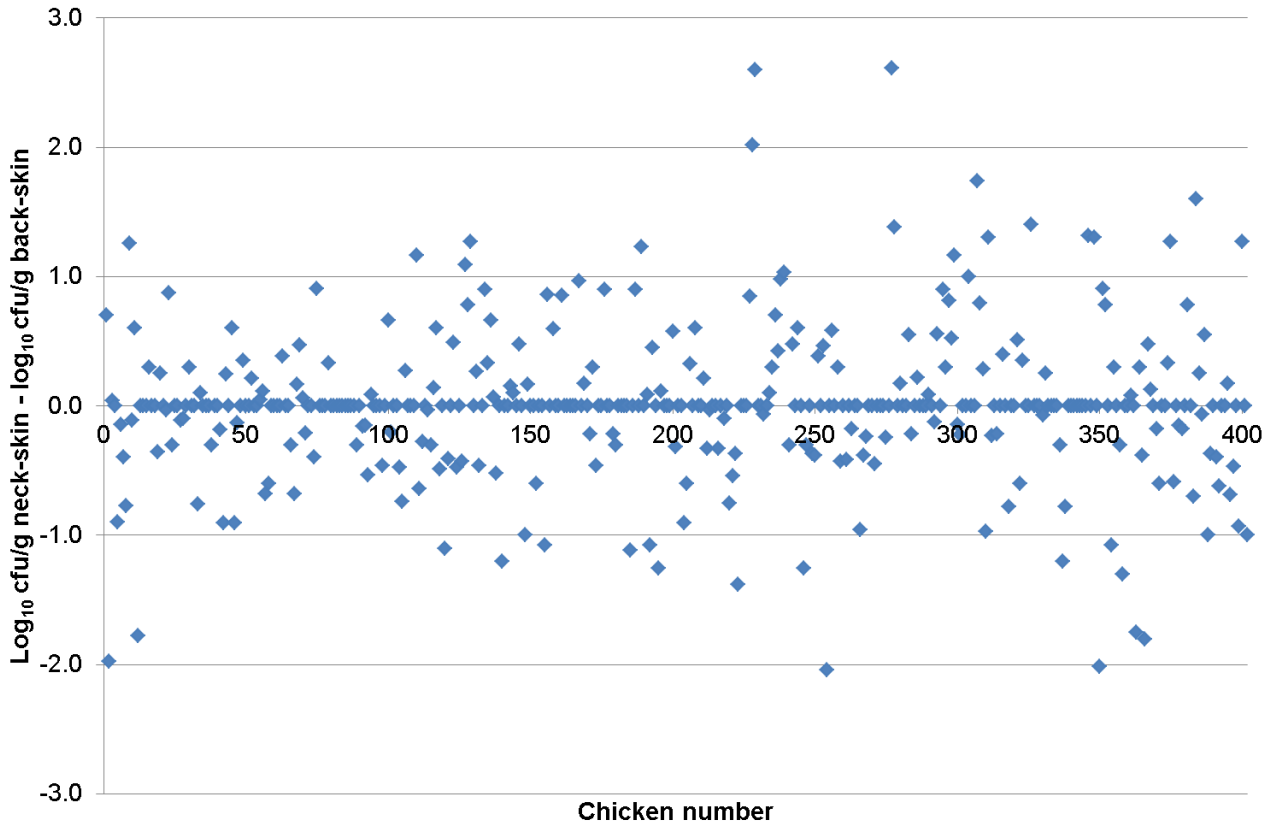

Figure 4. Distribution of differences in cfu of *Campylobacter* spp. in neck-skin and back-skin samples from 416 retail chickens

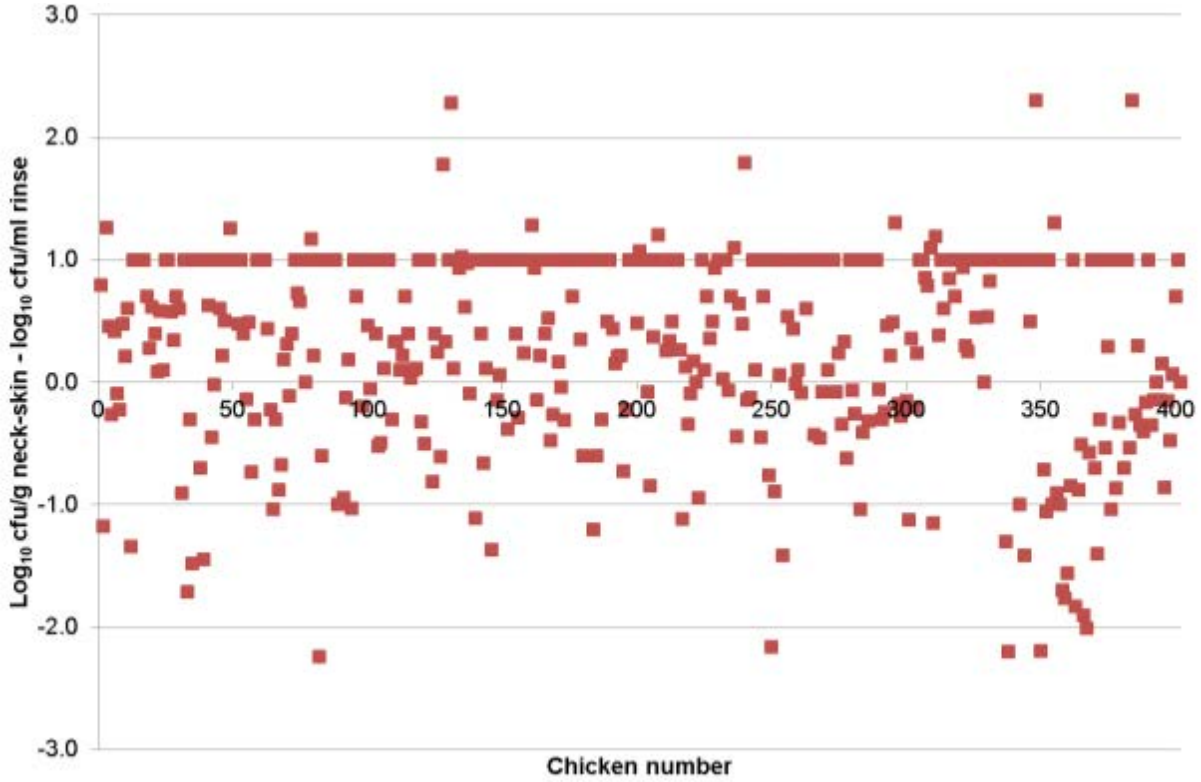

Figure 5. Distribution of differences in cfu of *Campylobacter* spp. in neck-skin and carcass-rinse samples from 416 fresh whole retail chickens

There was also a reasonable agreement in the number of *Campylobacter* cfu in back-skin and carcass-rinse samples (Table 19; Figure 6) and a McNemar test did not detect any significant difference in the proportion of chicken with > 1000 cfu of *Campylobacter* per g or ml between back-skin and carcass-rinse samples.

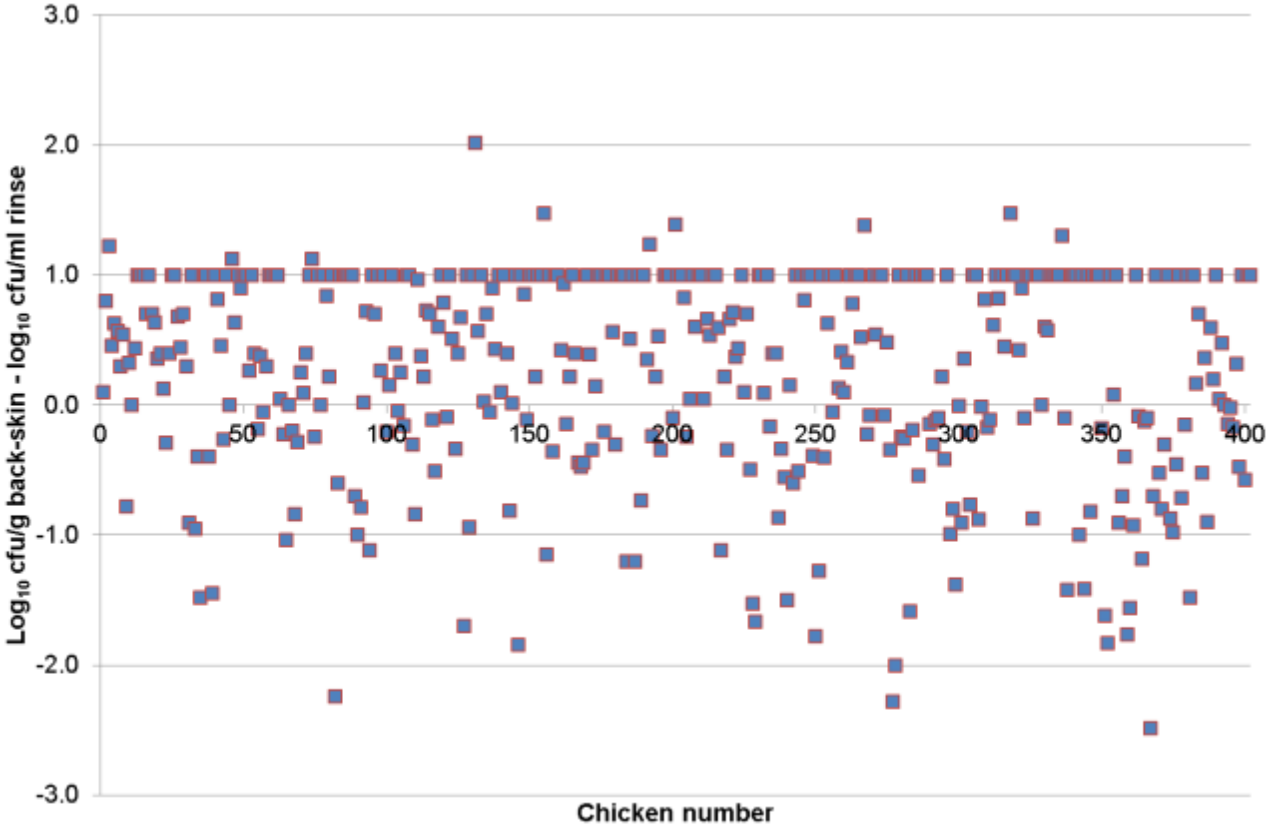

Figure 6. Distribution of differences in cfu of *Campylobacter* spp. in back-skin and carcass-rinse samples from 416 retail chickens

Table 19. *Campylobacter* spp. levels in back-skin and carcass-rinse samples from 416 retail chickens

		Number of chickens with level of *Campylobacter* in back-skin sample		
	Log_{10} *Campylobacter* per g or ml chicken	**< 1.15**	**1.15 – 3**	**> 3**
Number of chickens with level of *Campylobacter* in carcass rinse sample	**< 1.15**	184	22	0
	1.15 – 3	33	149	6
	> 3	1	15	6

The percentage of samples with > 1000 cfu per g (or ml) was 5.0 % (95% CI: 3.2 to 7.6 %) for neck-skin samples, 2.9 % (95% CI: 1.5 - 5.0 %) for back-skin samples and 5.3 % (95% CI: 3.3 - 7.9 %) for carcass-rinse samples.

In the isolates from the chicken neck skin samples that underwent speciation testing (n = 190); *C. jejuni* alone was found in 92.1 %, *C. coli* alone in 6.8 % and both species in 1.0 % (Table 20).

Table 20. *Campylobacter jejuni* and *C. coli* isolates from chicken neck-skin samples detected in method comparison trial

Species detected	No. of samples	% of total samples speciated (n = 190)
***C. jejuni* only**	175	92.1
***C. coli* only**	13	6.8
C. jejuni* and *C. coli	2	1.0

The neck-skin samples taken in phase 1 were 25 g samples which contained neck skin only or neck-skin supplemented with varying levels of breast skin. The samples taken in phase 2 were samples containing 10 g (or less) of neck skin alone. The $\log_{10}$ cfu of *Campylobacter* in the 25 and 10 g sample categories appeared to show a similar distribution (Figure 7). However, results from a regression model provided evidence that the proportion of neck skin/ breast skin in the samples had an effect on the measured contamination (p-value 0.02). After controlling for (removing) this effect, there was no evidence (p-value 0.4) to suggest a difference in the measured level of contamination between the 25 and the 10 g samples.

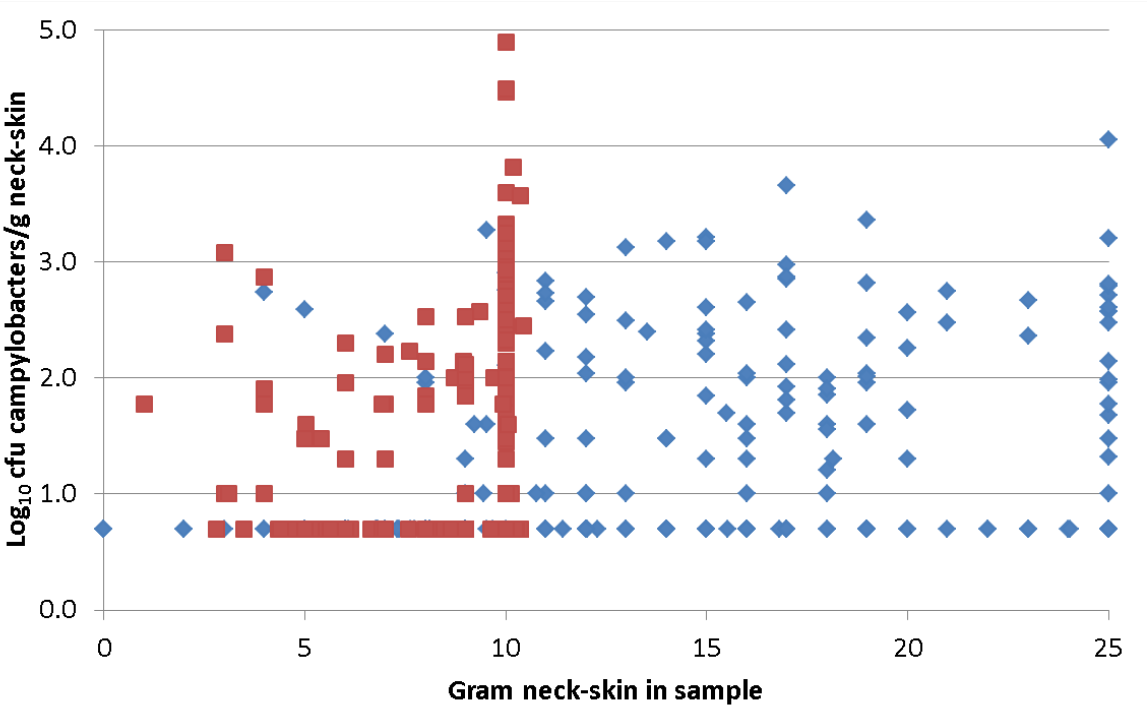

Figure 7. Counts of *Campylobacter* spp. per g neck-skin in relation to g neck-skin in sample and total sample weight (total sample weight = up to 10 g ■ ; = 25 g: ◆).

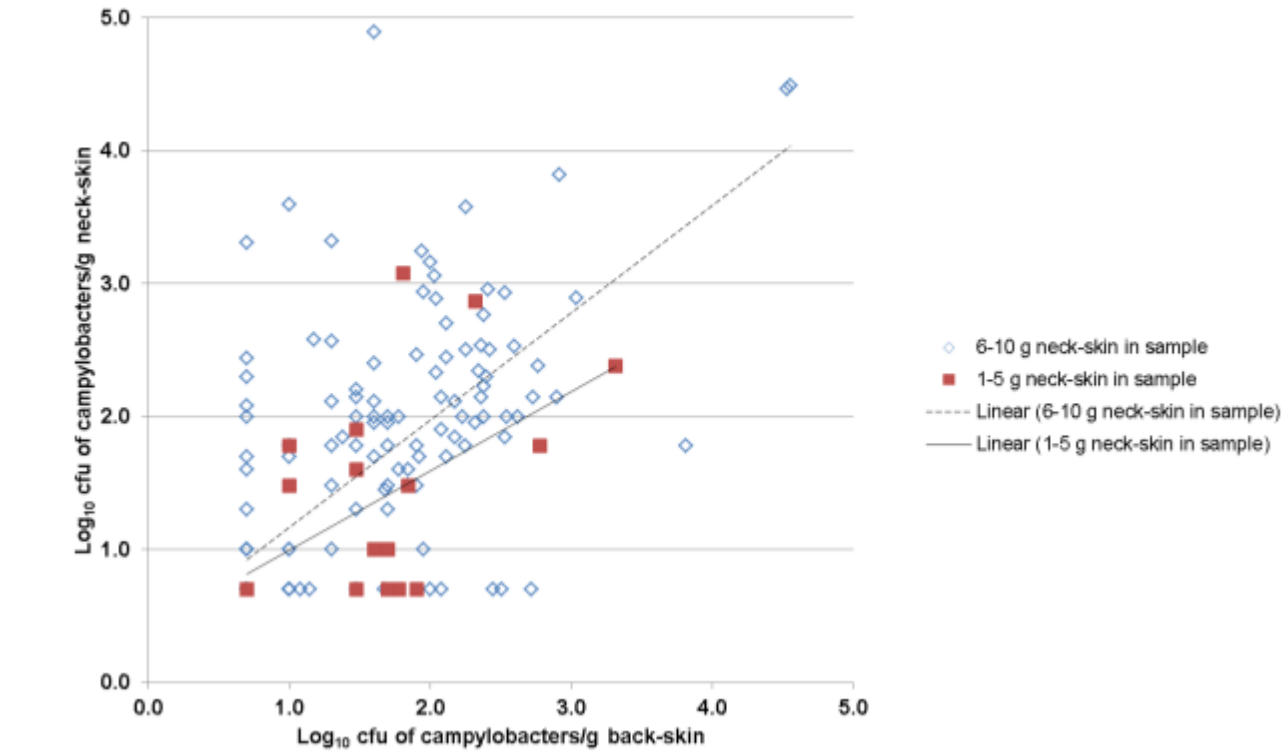

Figure 8. Counts of *Campylobacter* spp. per g neck-skin sample in relation to g neck-skin in samples with up to 10 g neck-skin.

Comparing the cfu per g in neck-skin samples with the cfu per g in back-skin samples for different total neck-skin weights possibly suggested a slight trend of lower cfu for neck-skin weights between 1-5 g compared to 6-10 g (Figure 8).

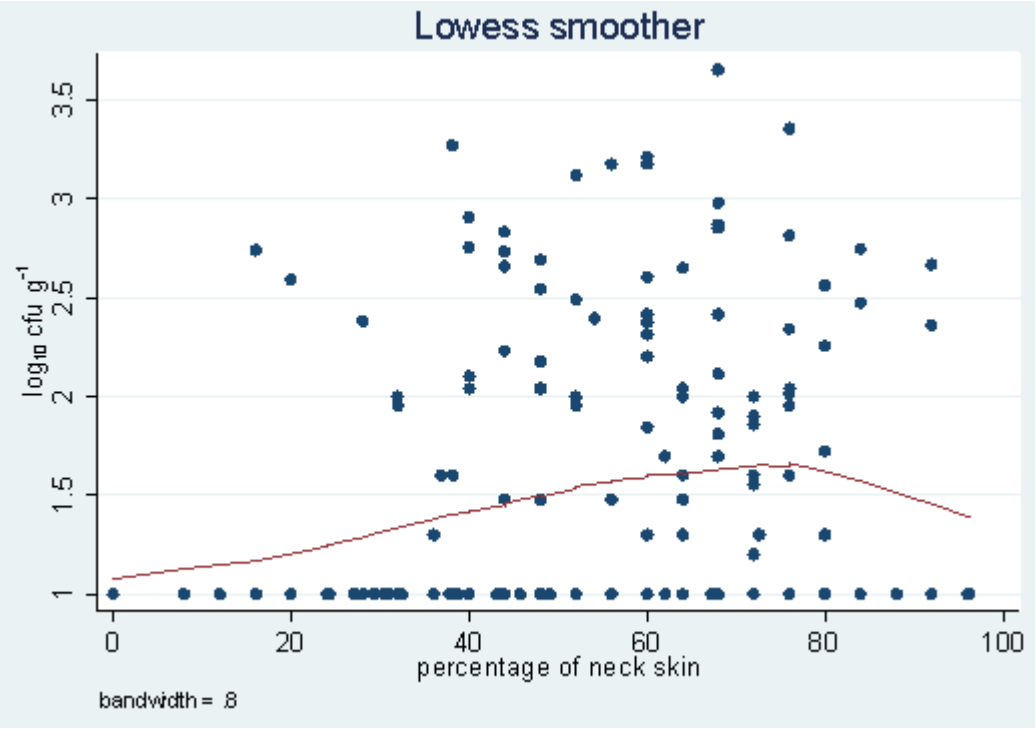

Figure 9. Level of *Campylobacter* spp. per g neck-skin in relation cfu per g back-skin for two neck-skin sample weight categories (total sample weight = up to 10 g).

However, the locally weighted scatter plot smoother of the measured $\log_{10}$ cfu per g against the g of neck skin analysed in chickens (Figure 9) where the sample consisted exclusively of 10 g or less of neck skin indicated that there is no evidence that the weight of neck skin analysed influenced the measured contamination.

4.0 Discussion

4.1 Survey results

In this survey the proportion of *Campylobacter* spp. in fresh whole UK produced chicken at retail was 61.3 %, whilst 11.4 % (95 % CI = 10.3 -12.6) of samples had >1000 cfu of campylobacters per g skin. In the previous survey year (FSA 2015), 73.3 % of chickens were contaminated and 19.4 % (95 % CI = 18.2 - 20.7) had >1000 cfu per g *Campylobacter* spp.. This could suggest that there is evidence of a significant reduction in contamination between the two survey years, however, further analysis and ongoing surveillance would determine whether this reflects a true sustained decline.

This work continued the testing of the outer packaging of retail chicken packs that was a novel aspect of Project FS241044 (FSA 2015). In 5.5 % of samples *Campylobacter* spp. were detected from the outer-packaging and while this was mostly at low levels, 1.0 % of samples had between 100-1000 *Campylobacter* spp. cfu per swab and 0.2 % had >1000 cfu per swab. The highest count recorded was 5740 cfu per swab.

There were moderate significant differences in the proportion of highly contaminated chickens between some major retailers. Compared against the industry average, Waitrose had the lowest proportion of highly contaminated chickens at 6.7 %, while ASDA and Sainsbury's had the highest proportions at 17.6 and 17.7 %, respectively. It would be reasonable to hypothesise that such differences could relate to a number of factors including chicken rearing factors (e.g. access to range, farm management and biosecurity levels), processing plant factors, weight/age of bird at slaughter, shelf-life remaining at testing and season. Accurate details were not available for all of these factors for all chickens tested. Nevertheless statistical analysis demonstrated that neither access to range during rearing, chicken weight at sale, days of shelf-life remaining, or season could explain the differences between retailers. Further studies would be needed to provide a more comprehensive understanding of the extent to which different processors can explain the differences between retailers. There was evidence that the approval number was associated with the level of campylobacter found on whole fresh chicken. However, the strong relationship between retailer and approval number precluded an investigation of approval number in the logistic regression analyses. Additionally, approval code is unlikely to feature in consumer purchasing decisions.

Whilst there was no evidence that free-range or organic chickens were more highly contaminated than standard birds, this finding should be treated with caution as low numbers of free-range and organic chickens were examined due to their low overall market share. Their corresponding confidence intervals were wide and would therefore only be able to verify very large differences. Nevertheless, a very similar finding was made in the first survey year.

The data suggested that a lower proportion of chickens had > 1000 cfu of campylobacter per g of skin during the winter months compared to the remaining study period. This result was also found in Project 241044 (PHE 2015), and the

prevalence of *Campylobacter* spp. in retail chicken, as determined by the presence/ absence test has also previously been associated with the time of year sampled (Meldrum 2005, CLASSP Project Team 2010, Hutchinson *et al*. 2006).

From the majority of chicken skin samples (83.0 %) *C. jejuni* only was isolated while *C. coli* only, was identified in 13.5 % of samples. A very similar species distribution was found in the previous survey year although in a slightly higher proportion of *C. jejuni* was found in the most recent survey year (PHE 2015). In an earlier FSA commissioned survey carried out in 2007 and 2008 (FSA 2009), the proportion of chickens (43 %) from which *C. jejuni* was isolated was considerably lower than in the current study. It is possible that this finding may relate to differences in the method of detection used. While this survey applied direct enumeration only, the 2007/2008 survey isolates were obtained using an enrichment method. In the CLASSP survey, where enrichment culture was used 62 % were *C. jejuni*, 32 % were *C. coli* and both species were detected in 6 % (CLASSP Project Team 2010). In the 2001 retail survey (FSA 2003), 25 % of isolates were *C. coli* only using an enrichment method. The proportion of human *C. jejuni* and *C. coli* strains in UK has been reported as approximately 90 % and 10 %, respectively.

Very similar proportions of the campylobacter-positive chicken skin and outer packaging samples harboured *C. jejuni* and/or *C. coli*. Furthermore, for the large majority of chicken packs where a *Campylobacter* spp. isolate was speciated from both the packaging and the skin sample, the same species was detected. This would be consistent with the outer packaging contamination originating from the chicken in the pack but without further characterisation (subtyping) of the isolates it is not possible to confirm this observation. Nevertheless, very similar results were found in the previous survey year and the data could suggest that these two species have a similar ability to contaminate and persist on outer packaging.

Recent slaughter house survey data for *Campylobacter* spp. on chicken carcasses tested after slaughter (and just before being put on retail sale) undertaken by the Animal and Plant Health Agency found a decrease in the proportion of contaminated carcases from approximately 79 % in 2012-13 to approximately 72 % in 2014-15 (FSA 2015c). This may suggest a recent downward trend that could also manifest itself in retail chickens but continued monitoring would be needed to verify this.

In summary, the proportion of chicken on sale in the UK that are contaminated with a high level of campylobacters is considerable but chickens from some retailers are less contaminated suggesting it is possible to achieve better control of *Campylobacter* spp. in chicken. Data from this part year and the previous survey year has demonstrated a significant decline in the level of highly contaminated fresh whole UK retail chicken. The FSA has indicated that the retail proxy for the proportion of highly contaminated retail chickens should be less than 7 % and continued monitoring will be required to demonstrate a sustained decline.

4.2 Human campylobacter infections in the UK

The EFSA Scientific Opinion published in 2011 (EFSA 2011) suggested that reducing the numbers of *Campylobacter* spp. on carcases by more than 99 % would reduce the public health risk by more than 90 %.

The reporting rate for *Campylobacter* spp. has decreased in the UK from 109.2 per 100,000 population in 2014 to 97.7 per 100,000 in 2015 (PHE 2016). The rate of reported *Campylobacter* infections in England has decreased to the lowest rate reported since 2008, and remains below the rate observed in Wales and Scotland (Figure 10). Northern Ireland continues to report rates lower than the rest of the United Kingdom. Wales is the only country to have reported a higher rate in 2015. Rates of reported infection in Scotland remain similar to that reported in recent years (Table 21).

Table 21. Number and rate of reported campylobacter infections in the United Kingdom and by country per 100,000 population, 2006-2015.

Year	England		Wales		Scotland		Northern Ireland		United Kingdom	
	N	Rate*	N	Rate*	n	Rate*	N	Rate*	n	Rate*
2006	43806	86.0	2942	98.5	4853	94.5	934	53.6	52535	86.4
2007	48622	94.6	3209	106.7	5190	100.4	881	50.0	57902	94.4
2008	47096	90.9	2795	92.4	4866	93.5	843	47.4	55600	89.9
2009	54438	104.3	3247	106.8	6398	122.3	974	54.3	65057	104.5
2010	59200	112.5	3388	111.1	6582	125.1	1036	57.4	70206	111.9
2011	60616	114.1	3911	127.7	6366	120.1	1171	64.5	72064	113.9
2012	61255	114.5	3789	123.3	6333	119.2	1205	66.1	72582	113.9
2013	55906	103.8	3134	101.7	6163	115.7	1349	73.7	66552	103.8
2014	58782	108.2	3712	120.1	6636	124.1	1415	76.9	70545	109.2
2015	51912	95.6	3795	122.7	6184	115.6	1320	71.7	63211	97.9

*rate per 100,000 population. Please note the 2015 figures for England and UK differ from that previously provided – this data has been finalised.

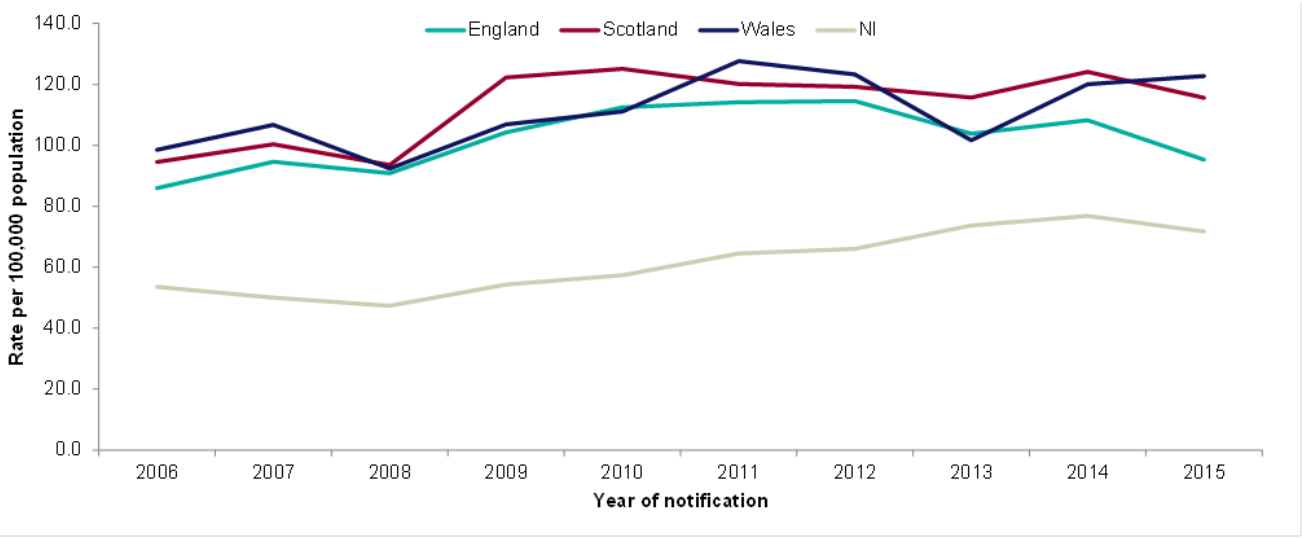

Figure 10. Rate of reported campylobacter infections by country per 100,000 population, 2006-2015

4.3 Method evaluation trial

The method evaluation trial demonstrated that *Campylobacter* spp. on fresh whole retail chicken can be enumerated using either neck-skin, back-skin or carcass rinse samples. Neck-skin and back-skin samples resulted in the most similar cfu per g, with 41 chickens (10 %) differing by > 1 $\log_{10}$. Although not very different, neck-skin and carcass-rinse samples identified more chickens with >1000 cfu per g or ml than the back-skin samples suggesting that the back-skin samples may fail to detect some highly contaminated chickens. Whilst a carcass-rinse sample detected *Campylobacter* spp. in more chickens than a neck-skin sample, a similar proportion of chickens with > 1000 cfu were identified using both these methods. Compared to the neck-skin method, the use of a carcass-rinse method required further resources due to the extra laboratory work required to confirm the presence of campylobacters in the additional (low level) contaminated carcasses. It is likely that carcass-rinse samples would also reflect contamination washed out from the carcass cavity (as well as surface contamination) unlike the skin reflecting only surface contamination. It is also possible that the carcass-rinse would be subject to more uncertainty compared to a skin sample as arguably the rinsing process may be more prone to experimental variation (e.g. through variations in the manual carcass washing technique). It is well known that it is possible to remove additional campylobacters by performing successive carcass-rinses (Jorgensen et al. 2002).
For monitoring purposes it would be difficult to meaningfully compare contamination with previous data if measured in different units, and this may result in weakening precision in the survey. Thus to continue to ascertain trends over time the neck-skin sample is best suited for this purpose.

There was, on average, a slightly lower level of cfu of *Campylobacter* spp. in samples with 10 g neck-skin (or less) compared to the 25 g neck/breast-skin samples. However, for a very high proportion of the chickens 25 g neck-skin was not available for testing potentially hampering equitable comparisons.

A limited number of chickens in the method trial had low amounts of neck-skin (< 5 g) available for testing; 7.5 % of samples had ≤ 5 g neck-skin, and 1.5 % had 2 g or less available for testing. Resolving this issue by adding in breast-skin to increase the sample weight to a total of 10 g may not result in a more equitable comparison between samples as breast-skin may be less contaminated than neck-skin. Analysis of samples consisting exclusively of 10 g or less of neck skin indicated that there is no evidence that the total sample weight of neck skin analysed influenced the measured contamination.

In the final protocol for survey year 3 (Appendix III), the neck-skin sample was maintained with a reduction in the weight of sample tested to 10 g pure neck-skin (using down to 5 g where < 10 g available) per chicken to minimise any sample bias in the retailer comparisons.

4.4 Conclusions

- The proportion of chicken on sale in the UK that are contaminated with a high level of campylobacter is considerable, but chickens from some retailers are less contaminated suggesting that it is possible to achieve better control of *Campylobacter* spp. in chicken.

- Data from this part year and the previous survey year has identified a significant decline in the level of highly contaminated fresh whole retail chicken in the UK.

- The FSA has indicated that the retail proxy for the proportion of highly contaminated retail chickens should be less than 7 % and continued monitoring will be required to demonstrate a sustained decline.

- The epidemiological data of human cases show a decrease in the reporting rate for *campylobacter* species overall for the UK by 11.5 per 100,000 population between 2014 and 2015. This reduction is most pronounced in England.

- The outcome of the method evaluation trial was to maintain testing of a neck-skin sample but with a reduction in the weight of sample tested to a maximum of 10 g (pure) neck-skin (allowing down to a 5 g sample where < 10 g neck-skin available) to ensure comparable samples from the large majority of chickens sampled.

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6.0 Appendices

6.1 Appendix I Main survey protocol and method comparison trial

- Appendix 6.1a: Main Survey Protocol
- Appendix 6.1b: Method Comparison Protocol
  - Appendix 6.1b-Appendix 1a-Method flow chart for Phase I
  - Appendix 6.1b-Appendix 1b-Method flow chart for Phase II
  - Appendix 6.1b-Appendix 2-Draft Protocol for the Campylobacter Retail Survey (Year 3 /4)

6.2 Appendix II Main survey year 2 data and method comparison data

- Appendix 6.2a: Main Survey Year 2 data for Q1-3 Data (FS102121)
- Appendix 6.2b: Raw data for method evaluation

6.3 Appendix III Survey year 3 and 4 protocol