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FSA Project FS102121

Year 2 Report

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

Frieda Jorgensen, Andre Charlett, Eve Arnold, Craig Swift, Bob Madden and Nicola C Elviss

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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.

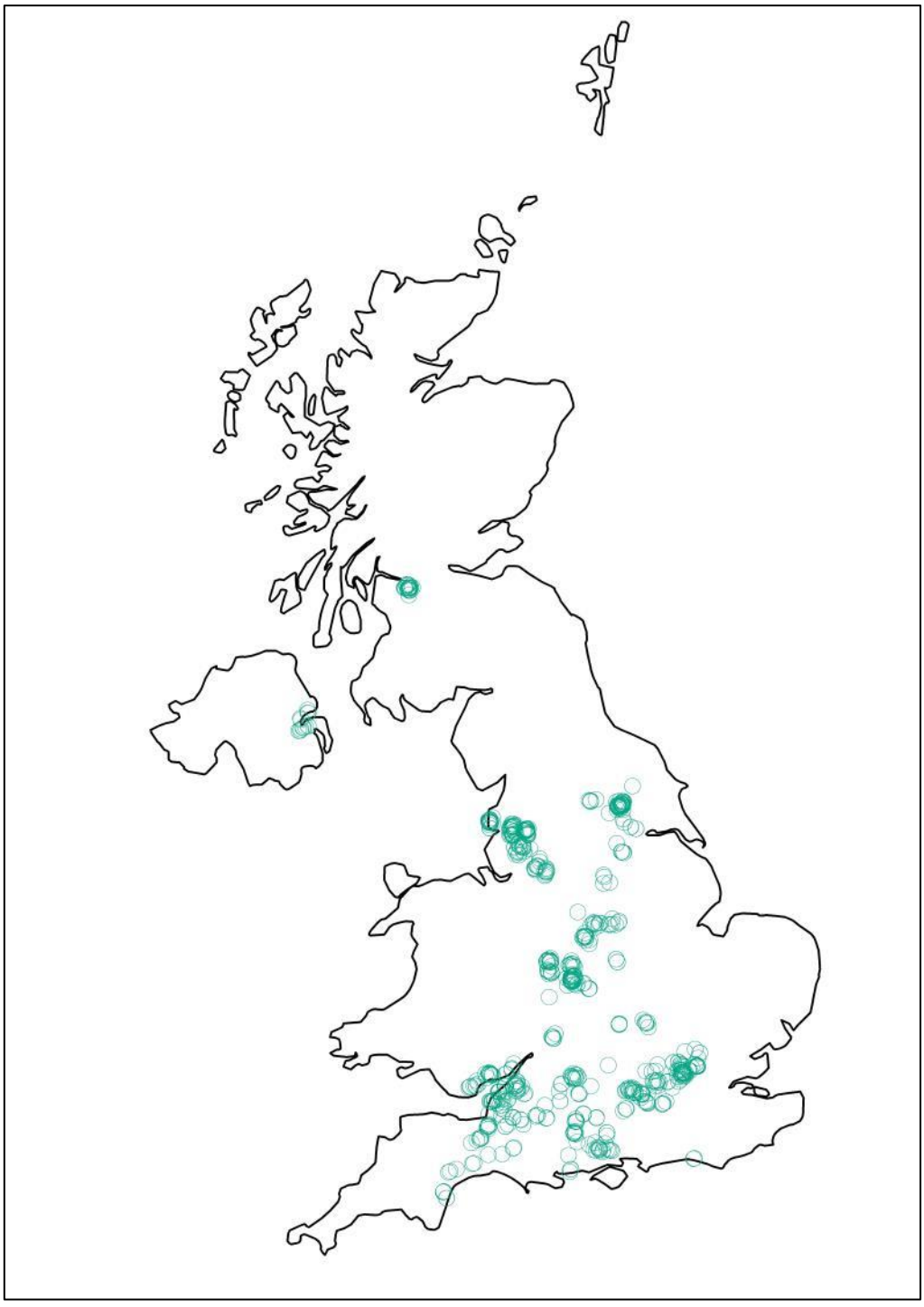

Figure 1. Geographical distribution of samples collected for the survey

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

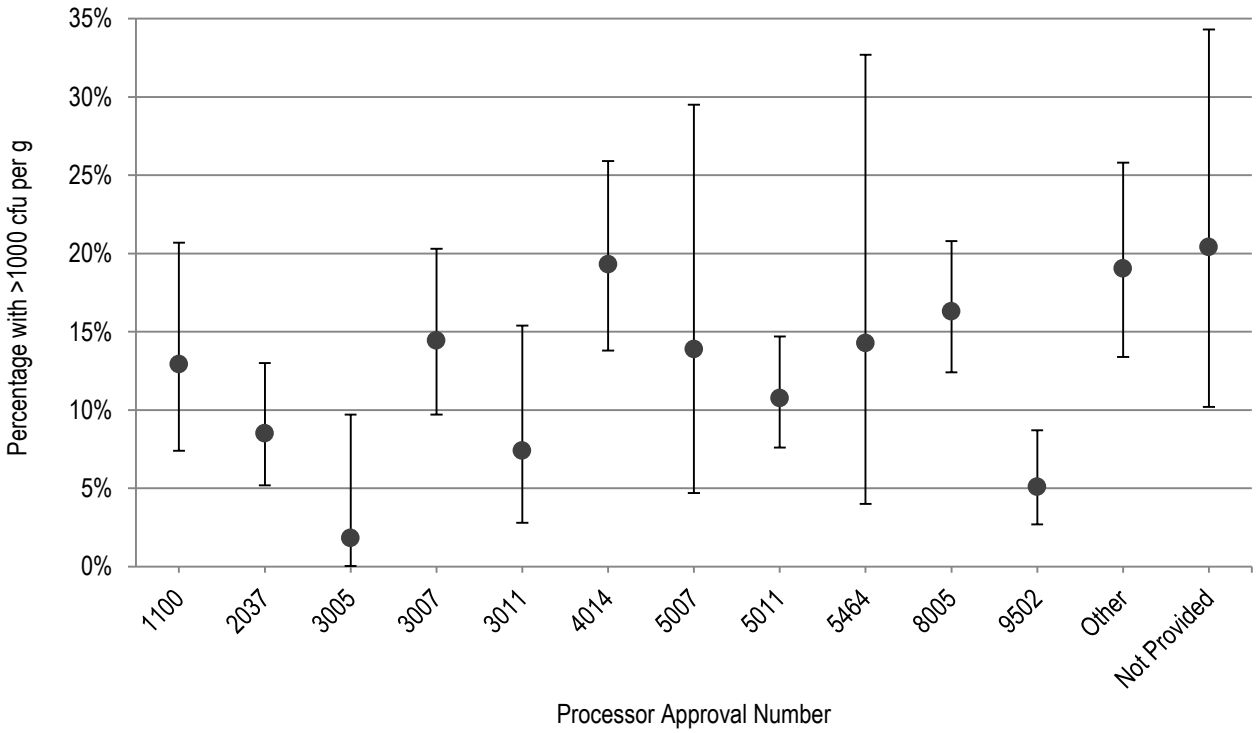

Figure 2. The percentage of chickens with >1000 cfu of campylobacters per g chicken skin in relation to processor approval number. Samples listed within the 'Other' code category had < 50 chickens from the processor sampled within the study. A list of approved premises codes can be found on the FSA website 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).

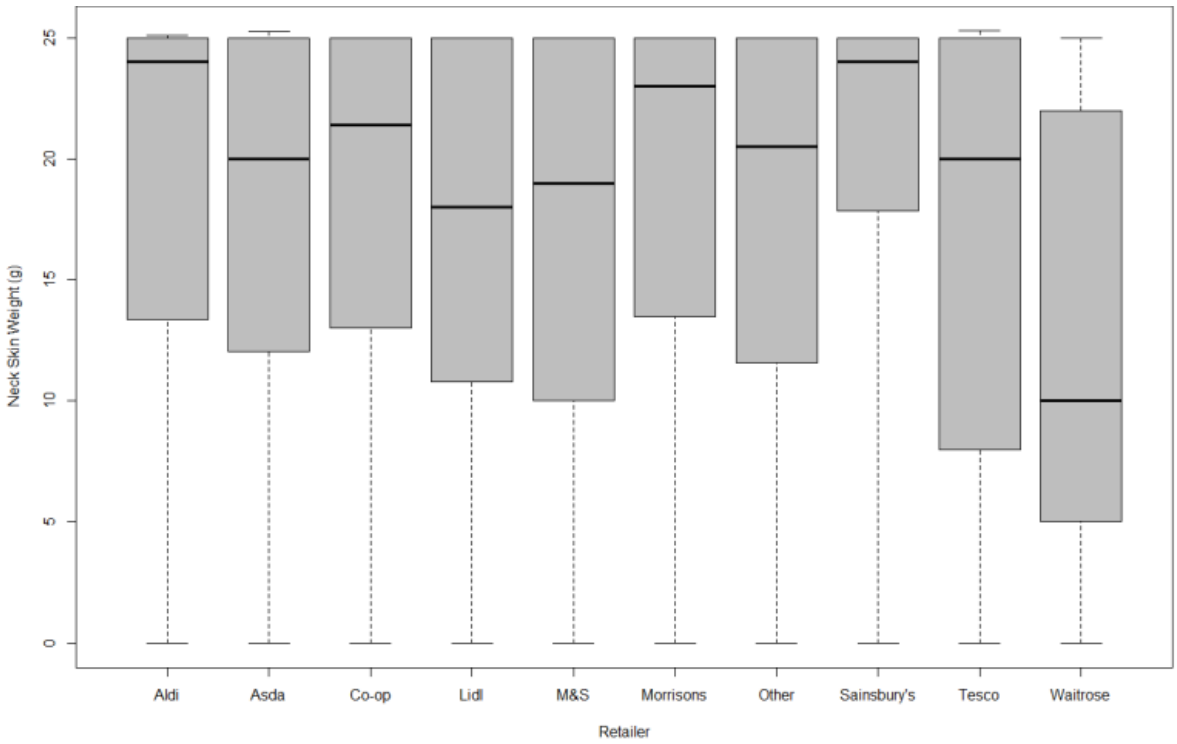

Figure 3. Chicken neck-skin weight in samples in relation to retailer (Quarters 1-2)

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

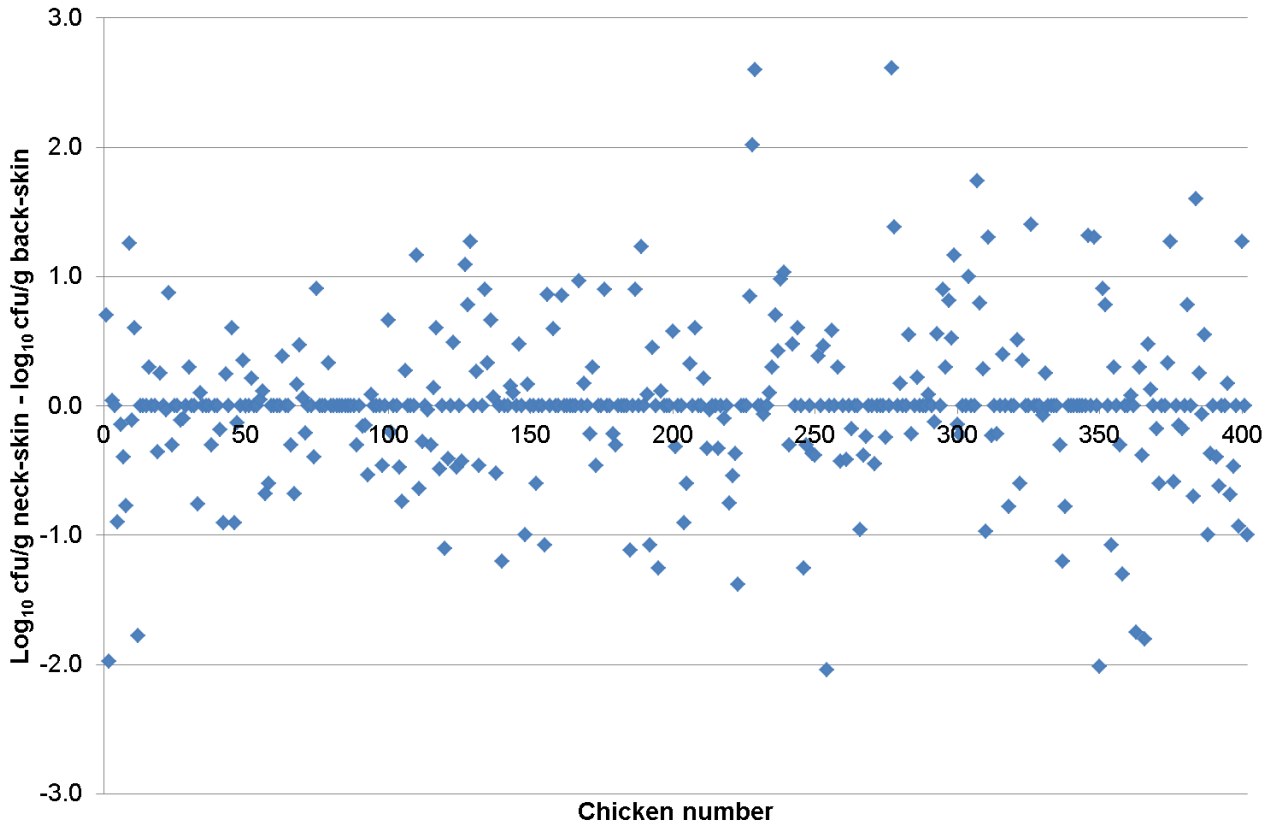

Figure 4. Distribution of differences in cfu of *Campylobacter* spp. in neck-skin and back-skin samples from 416 retail chickens

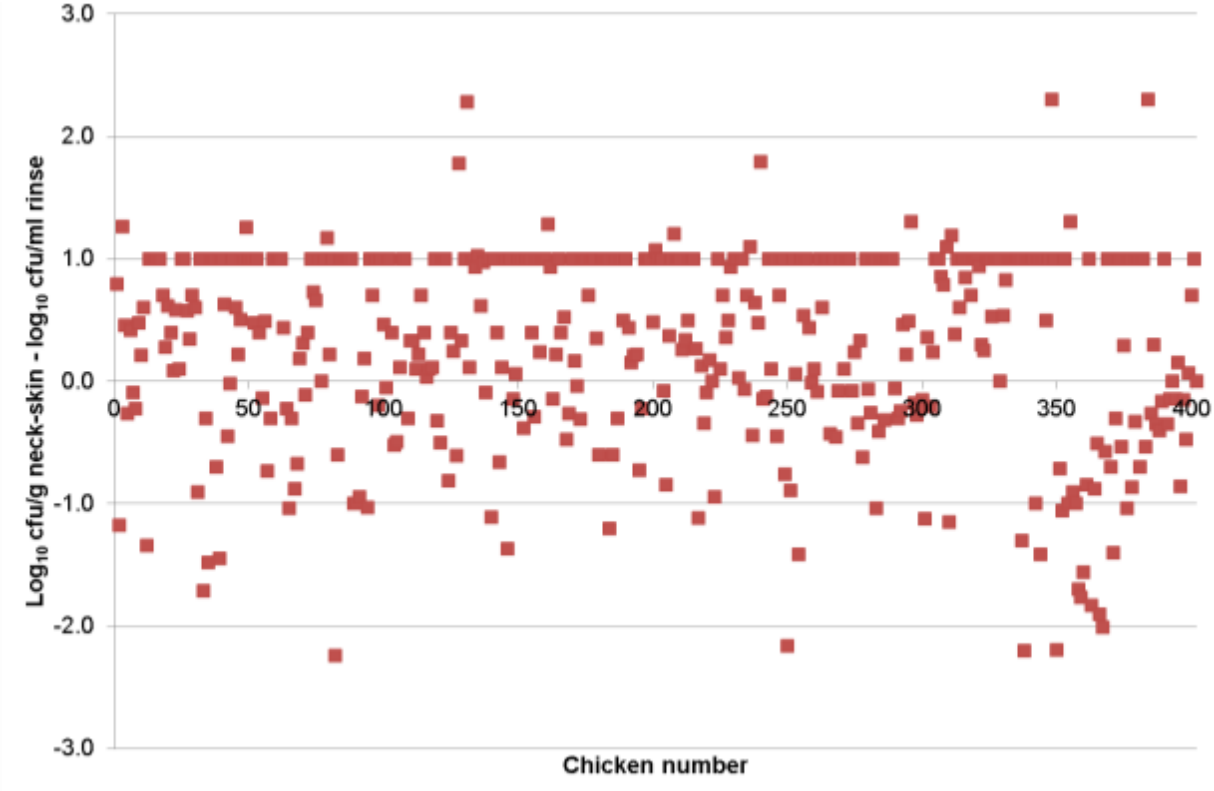

Figure 5. Distribution of differences in cfu of *Campylobacter* spp. in neck-skin and carcass-rinse samples from 416 fresh whole retail chickens

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.

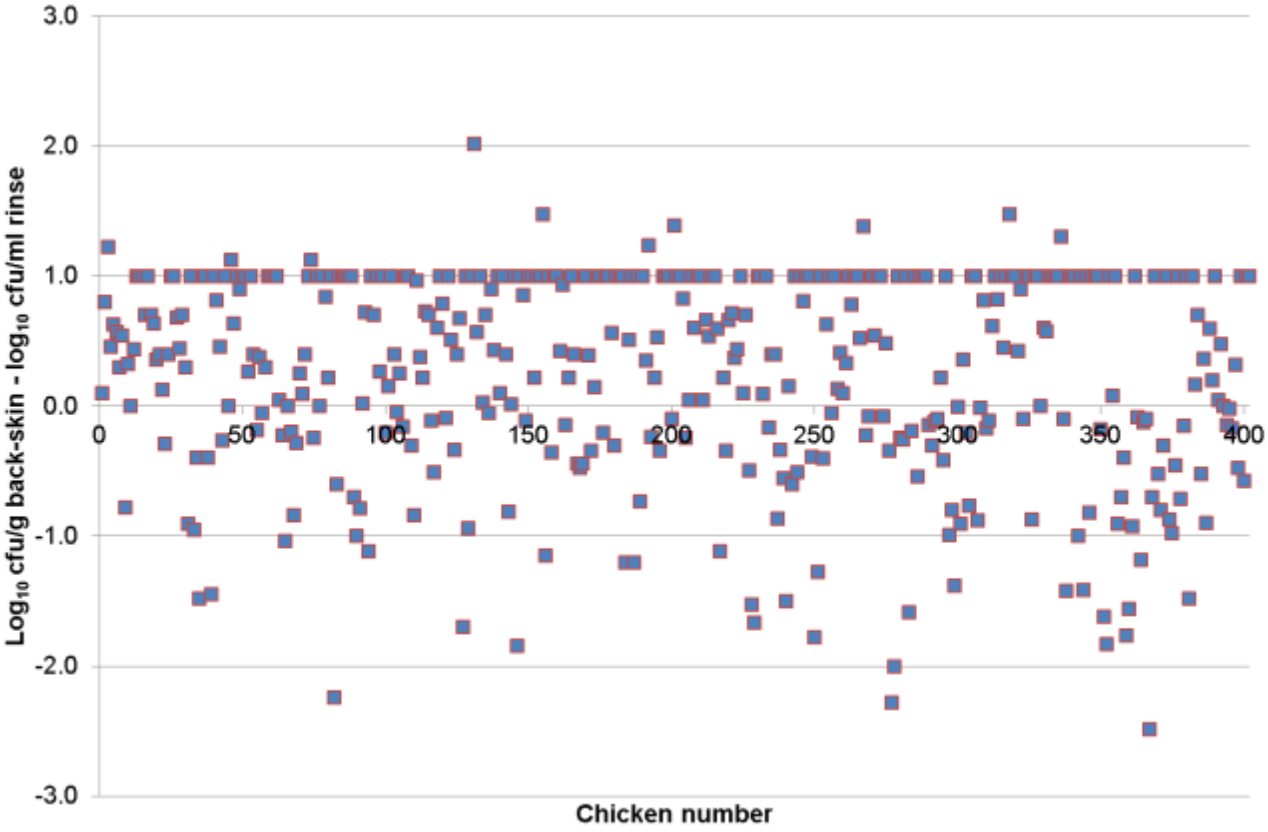

Figure 6. Distribution of differences in cfu of *Campylobacter* spp. in back-skin and carcass-rinse samples from 416 retail chickens

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.

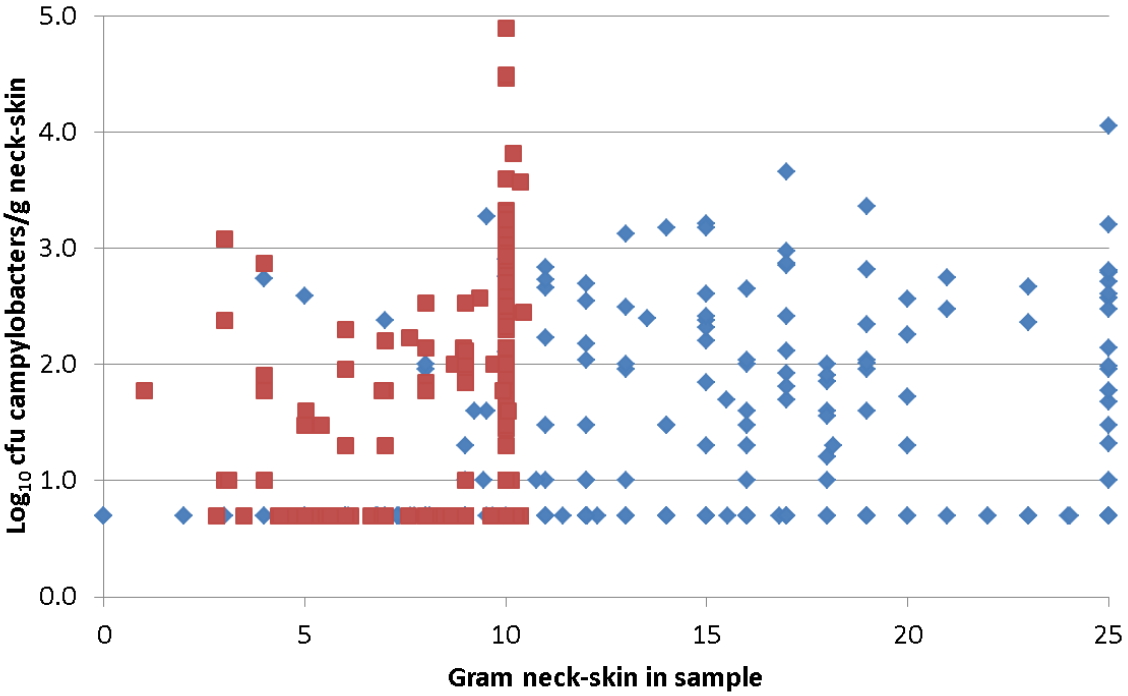

Figure 7. Counts of *Campylobacter* spp. per g neck-skin in relation to g neck-skin in sample and total sample weight (total sample weight = up to 10 g ■ ; = 25 g: ◆).

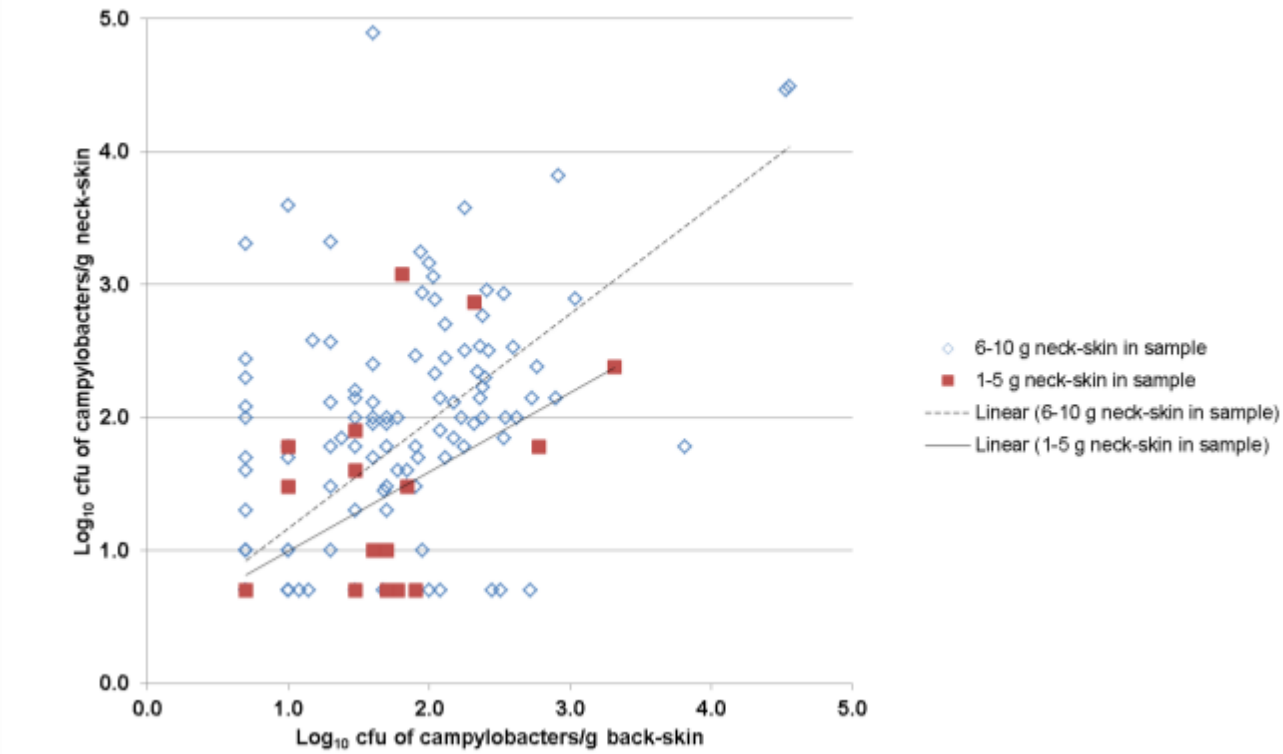

Figure 8. Counts of *Campylobacter* spp. per g neck-skin sample in relation to g neck-skin in samples with up to 10 g neck-skin.

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).

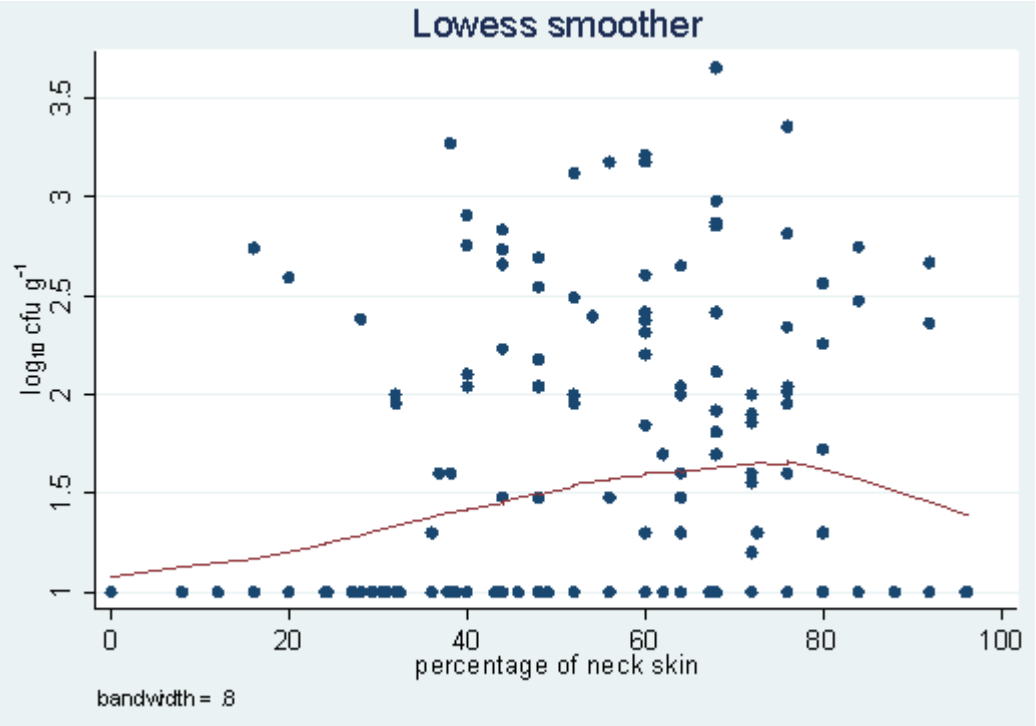

Figure 9. Level of *Campylobacter* spp. per g neck-skin in relation cfu per g back-skin for two neck-skin sample weight categories (total sample weight = up to 10 g).

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.

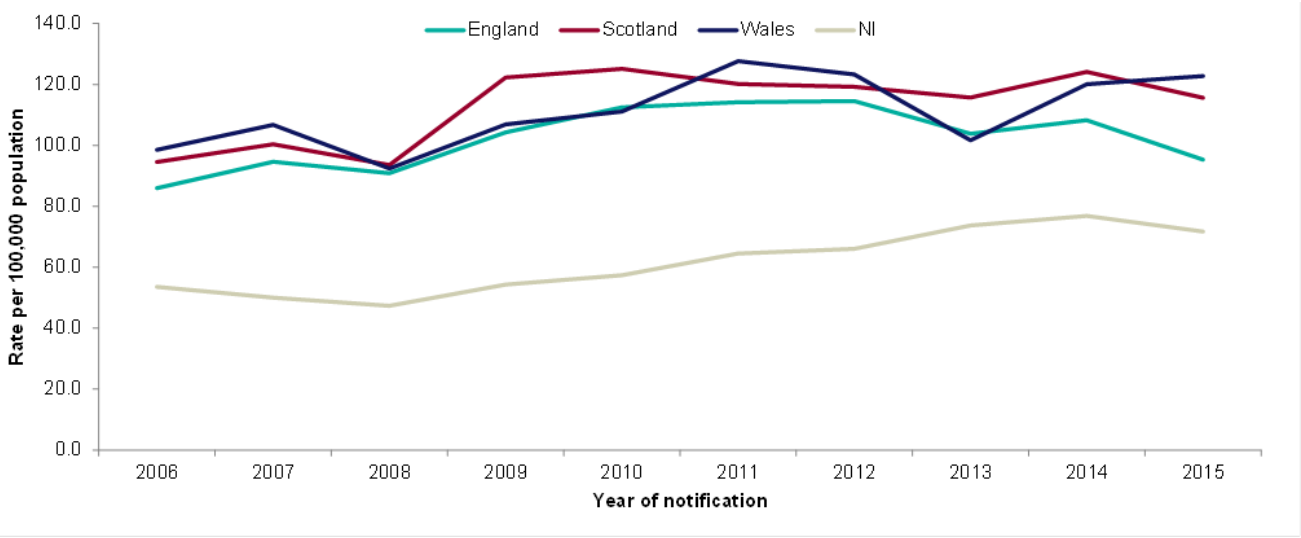

Figure 10. Rate of reported campylobacter infections by country per 100,000 population, 2006-2015

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.

5.0 References

Allen, V.M., Bull, S.A., Corry, J.E., Domingue, G., Jørgensen, F., Frost, J.A., Whyte, R., Gonzalez, A., Elviss, N. and Humphrey, T.J. (2007). *Campylobacter* spp. contamination of chicken carcasses during processing in relation to flock colonisation. Int. J. Food Microbiol. 113:54-61.

Anonymous. (2006) International Organisation for Standardisation ISO/TS 10272-2. Microbiology of food and animal feeding stuffs – horizontal method for the detection and enumeration of *Campylobacter* – Part 2: colony count technique. International Organisation for Standardisation, Geneva.

Best EL, Powell EJ, Swift C, Grant KA, Frost JA. (2003). Applicability of a rapid duplex real-time PCR assay for speciation of *Campylobacter jejuni* and *Campylobacter coli* directly from culture plates. FEMS Microbiol Lett. 229:237-241.

Blankenship, L.C., Craven, S.E. (1982) *Campylobacter jejuni* survival in chicken meat as a function of temperature. Appl Environ Microbiol. 44:88-92.

Borck, B., H. Stryhn, A. K. Ersboll, and K. Pedersen. (2002). Thermophilic *Campylobacter* spp. in turkey samples: evaluation of two automated enzyme immunoassays and conventional microbiological techniques. J. Appl. Microbiol. 92:574-582.

Bull, S.A., Allen, V.M., Domingue, G., Jørgensen, F., Frost, J.A., Ure, R., Whyte, R., Tinker, D., Corry, J.E., Gillard-King, J. and Humphrey, T.J. (2006). Sources of Campylobacter spp. colonizing housed broiler flocks during rearing. Appl Environ Microbiol. 72:645-652.

CLASSP Project Team (2010) LACORS/HPA Coordinated Local Authority Sentinel Surveillance of Pathogens (CLASSP) Final Report.

Danis, K., Di Renzi, M., O'Neill, W., Smyth, B., McKeown, P., Foley, B., Tohani, V. and Devine, M. (2009) Risk factors for sporadic *Campylobacter* infection: an all-Ireland case-control study. Euro Surveill. 14. pii: 19123.

Davis, M.A. and Conner, D.E. (2007) Survival of *Campylobacter jejuni* on Poultry Skin and Meat at Varying Temperatures. *Poultry Science* 86:765-767.

Dufrenne, J., Ritmeester, W., Delfgou-van Asch, E., van Leusden, F. and de Jonge, R. (2001). Quantification of the contamination of chicken and chicken products in The Netherlands with *Salmonella* and *Campylobacter*. J. Food Prot. 64, 538-541

European Food Safety Authority (EFSA). (2009). Scienitifc Opinion on Quantification of the risk posed by broiler meat to human campylobacteriosis in the EU (adopted 9 December 2009) http://www.efsa.europa.eu/en/scdocs/scdoc/1437.htm

European Food Safety Authority (EFSA). (2011). Scientific Opinion on *Campylobacter* in broiler meat production: control options and performance objectives and/or targets at different stages of the food chain. EFSA Journal 9:2105.

European Food Safety Authority (EFSA). (2010a). Analysis of the baseline survey on the prevalence of *Campylobacter* in broiler batches and of *Campylobacter* and *Salmonella* on broiler carcasses in the EU, 2008; Part A: *Campylobacter* and *Salmonella* prevalence estimates. EFSA J. 8:1503.

European Food Safety Authority (EFSA). (2010b). Analysis of the baseline survey on the prevalence of *Campylobacter* in broiler batches and of *Campylobacter* and *Salmonella* on broiler carcasses, in the EU, 2008; Part B: Analysis of factors associated with *Campylobacter* colonisation of broiler batches and with *Campylobacter* contamination of broiler carcasses; and investigation of the culture method diagnostic characteristics used to analyse broiler carcass samples. EFSA J. 8:1522.

Figueroa, G., Troncoso, M., López, C., Rivas, P. and Toro, M. (2009). Occurrence and enumeration of *Campylobacter* spp. during the processing of Chilean broilers. BMC Microbiol. 9:94.

Food Standards Agency (2003). UK-wide Survey of *Salmonella* and *Campylobacter* Contamination of Fresh and Frozen Chicken on Retail Sale. Available at: http://www.food.gov.uk/multimedia/pdfs/campsalmsurvey.pdf (Last accessed 28 July 2015)

Food Standards Agency (2009). FSA report for the UK survey of *Campylobacter* and *Salmonella* contamination of fresh chicken at retail sale. FSA Project B18025. thttp://tna.europarchive.org/20140306205048/http://multimedia.food.gov.uk/multimedia/pdfs/fsis0409.pdf (Last accessed 28 July 2015)

Food Standards Agency (2010). The joint government and industry target to reduce campylobacter in UK produced chickens by 2015. Available at: http://www.food.gov.uk/multimedia/pdfs/campytarget.pdf (Last accessed 28 July 2015)

Food Standards Agency (2013) Open Board – 11 September 2013 A refreshed strategy to reduce campylobacteriosis from poultry. Available at: http://www.food.gov.uk/sites/default/files/multimedia/pdfs/board/board-papers-2013/fsa-130904.pdf (Last accessed 28 July 2015)

Food Standards Agency (2015a). ACT: Acting on *Campylobacter* Together http://www.food.gov.uk/news-updates/campaigns/campylobacter/ (Last accessed 28 July 2015)

Food Standards Agency (2015b). A UK wide microbiological survey of *Campylobacter* contamination in fresh whole chilled chickens at retail sale (Year 2/ 3/ 4). Available at: http://www.food.gov.uk/sites/default/files/Campylobacter%20Retail%20Survey%20Year%202%20protocol%20%28final%29.pdf (Last accessed 19 September 2016).

Food Standards Agency (2015c). FSA Board meeting 15 July 2015: Update on the *Campylobacter* Campaign http://www.food.gov.uk/sites/default/files/fsa150705.pdf (Last accessed 28 July 2015)

Food Standards Agency (2015d). *Campylobacter* survey: cumulative results from the full 12 months (Q1 - Q4). Available at: http://www.food.gov.uk/science/microbiology/campylobacterevidenceprogramme/retail-survey#toc-1 (Last accessed 28 July 2015)

Food Standards Agency (2016). Campylobacter contamination in fresh whole chilled UK-produced chickens at retail: January – March 2016: http://www.food.gov.uk/sites/default/files/campy-survey-report-jan-mar-2016.pdf

Friedman, C.R., Hoekstra, R.M., Samuel, M., Marcus, R., Bender, J., Shiferaw, B., Reddy, S., Ahuja, S.D., Helfrick, D.L., Hardnett, F., Carter, M., Anderson, B. and Tauxe, R.V.; Emerging Infections Program FoodNet Working Group. (2004). Risk factors for sporadic *Campylobacter* infection in the United States: A case-control study in FoodNet sites. Clin. Infect. Dis. 38 Suppl 3:S285-96.

Fukushima H, Katsube K, Hata Y, Kishi R. and Shimada S. (2007). Rapid Separation and Concentration of Food-borne Pathogens in Food Samples Prior to Quantification by Viable Count and Real-time PCR. Appl. Environ. Microbiol. 73:92-100.

Grigoriadis, S.G, Koidis, P.A., Vareltzis, K.P. and Batzios, C.A. (1997) Survival of *Campylobacter jejuni* Inoculated in Fresh and Frozen Beef Hamburgers stored under Various Temperatures and Atmospheres Journal of Food Protection 8: 883-1012/ 903-907

Habib, I., Sampers, I., Uyttendaele, M., Berkvens, D. and De Zutter, L. (2008). Baseline data from a Belgium-wide survey of *Campylobacter* species contamination in chicken meat preparations and considerations for a reliable monitoring program. Appl. Environ. Microbiol. 74:5483-5489.

Hong, Y., Berrang, M. E., Liu T., Hofacre, C.L., Sanchez, S., Wang, L. and Maurer, J.J. (2003). Rapid detection of *Campylobacter coli*, *C. jejuni*, and *Salmonella enterica* on poultry carcasses by using PCR-enzyme-linked immunosorbent assay. Appl Environ Microbiol. 69:3492-3499.

Hutchison, M. L., Walters, L. D., Allen, V. M., Mead, G. C. and Howell, M. (2006). Measurement of *Campylobacter* numbers on carcasses in British poultry slaughterhouses. J. Food Prot 69:421-424.

Jasson, V., Sampers, I., Botteldoorn, N., López-Gálvez, F., Baert, L., Denayer, S., Rajkovic, A., Habib, I., De Zutter, L., Debevere, J. and Uyttendaele, M. (2009). Characterization of *Escherichia coli* from raw poultry in Belgium and impact on the detection of *Campylobacter jejuni* using Bolton broth. Int J Food Microbiol. 135:248-53.

Jorgensen, F., Bailey, R., Williams, S., Henderson, P., Wareing, D.R., Bolton ,F.J., Frost, J.A., Ward, L. and Humphrey, T.J. (2002). Prevalence and numbers of *Salmonella* and *Campylobacter* spp. on raw, whole chickens in relation to sampling methods. Int. J. Food Microbiol. 76:151-64.

Meldrum, R. J., I. D. Tucker., R. M. and Smith, C. (2005). Three-year surveillance programme in Wales and Northern Ireland examining the prevalence of *Campylobacter* and *Salmonella* in retail raw chicken. J Food Prot. 68:1447-1449.

Melero, B., Cocolin L., Rantsiou K., Jaime I. and Rovira J. (2011). Comparison between conventional and qPCR methods for enumerating *Campylobacter jejuni* in a poultry processing plant. Food Microbiol. 28:1353-1358.

Mullner, P., Jones, G., Noble, A., Spencer, S.E., Hathaway, S. and French, N.P. (2009). Source Attribution of Food-borne Zoonoses in New Zealand; a modified Hald Model. Risk Anal. 29:970-984.

Oyarzabal, O. A., Macklin, K. S., Barbaree, J. M. and Miller, R.S. (2005). Evaluation of agar plates for direct enumeration of *Campylobacter* spp. from poultry carcass rinses. Appl. Environ. Microbiol. 71:3351-3354.

Public Health England. 2015. A Microbiological survey of campylobacter contamination in fresh whole UK produced chilled chickens at retail sale (2014-15). https://www.food.gov.uk/sites/default/files/campylobacter-retail-survey-final-report.pdf. (Last accessed 22 January 2016)

Public Health England (2016). Unpublished data.

Purnell, G., K. Mattick, and T. Humphrey. (2004). The use of “hot wash” treatments to reduce the number of pathogenic and spoilage bacteria on raw retail poultry. J. Food Eng. 62:29-36

Reich F and Atanassova V. *et al*. (2008). Effects of *Campylobacter* numbers in caeca on the contamination of broilers carcasses with *Campylobacter*. International Journal of Food Microbiology. 127:116-120.

Rosenquist, H., Nielsen, N. L., Sommer, H. M., Norrung, B. and Christensen, B. B. (2003). Quantitative risk assessment of human campylobacteriosis associated with thermophilic *Campylobacter* species in chickens. Int. J. Food Microbiol. 83:87-103. Int J Food Microbiol. 2007 Sep 15;118(2):201-13. Epub 2007 Aug 1.

Rosenquist, H., Bengtsson A. and Hansen, T.B. (2007) A collaborative study on a Nordic standard protocol for detection and enumeration of thermotolerant Campylobacter in food (NMKL 119, 3. Ed., 2007).

Sampers, I., Habib, I., Berkvens, D., Dumoulin, A., Zutter, L.D. and Uyttendaele, M. (2008). Processing Practices Contribute to *Campylobacter* Contamination in Belgian Chicken Meat Preparation. Int. J. Food Microbiol. 128:297-303.

Scherer, K., Bartelt, E., Sommerfeld, C. and Hildebrandt, G. (2006). Comparison of different sampling techniques and enumeration methods for the isolation and quantification of *Campylobacter* spp. in raw retail chicken legs. Int J Food Microbiol. 108:115-119.

Sheppard S.K., Dallas J.F., Strachan N.J.C., MacRae M., McCarthy N.D., Wilson D.J., Gormley F.J., Falush D., Ogden ID, Maiden MCJ and K.J. Forbes (2009). *Campylobacter* genotyping to determine the source of human infection. Clin. Infec. Dis. 48:1072-1078.

Strachan N.J.C. and Forbes K.J. (2010). The growing UK epidemic of human campylobacteriosis. Lancet 376:665–667.

Tam, C.C., Higgins, C.D., Neal, K.R., Rodrigues, L.C., Millership, S.E., O'Brien, S.J. (2009). *Campylobacter* Case Control Study Group. Emerg. Infect. Dis. 15:1402

Tam CC, Rodrigues LC, Viviani L, Dodds JP, Evans MR, Hunter PR, Gray JJ, Letley LH, Rait G, Tompkins DS and O'Brien SJ (2012). Longitudinal study of infectious intestinal disease in the UK (IID2 study): incidence in the community and presenting to general practice. Gut 61:69-77.

Wolffs, P., Norling, B., Hoorfar, J., Griffiths, M. and Radstrom, P. (2005). Quantification of *Campylobacter* spp. In chicken rinse samples by using flotation prior to real-time PCR. Appl. Environ. Microbiol. 71:5759-5764.

6.0 Appendices

6.1 Appendix I Main survey protocol and method comparison trial

i. Appendix 1: Main survey Protocol
ii. Appendix 1: Method Comparison Protocol

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

i. Appendix 2: Quarters 1-3 Raw Data
ii. Appendix 2: Raw Data for Method Evaluation

6.3 Appendix III Survey year 3 and 4 protocol

A UK WIDE MICROBIOLOGICAL SURVEY OF CAMPYLOBACTER CONTAMINATION IN FRESH WHOLE CHILLED CHICKENS AT RETAIL SALE (Year 2/ 3/ 4)

PROTOCOL

Revised July 2015

Updated 07.07.2015

CONTENTS

ABBREVIATIONS

CCDA-	Charcoal Cefoperazone Deoxycholate Agar
DH -	Department of Health
FSA -	Food Standards Agency
h -	Hour(s)
PHE -	Public Health England
ISO-	International Standards Organisation
LGP-	Laboratory of Gastrointestinal Pathogens
mL -	Millilitres
mm -	Millimetres
s -	Seconds
MS-	Microbiological Services
UKAS-	United Kingdom Accreditation Service

OUTLINE

Background

1. The Food Standards Agency has a key role in preventing foodborne illnesses. The Strategic Plan aims to reduce foodborne disease further and has set a target to reduce Campylobacter contamination in raw chicken.

   Campylobacter is the most prominent bacterium associated with foodborne disease within the United Kingdom. Foodborne Campylobacter is estimated to make more than 280,000 people ill each year in the UK and is the biggest cause of food poisoning. An EFSA Opinion[1] stated that up to 80% of cases can be attributed to raw poultry meat. It is hoped that by reducing the number of highly positive birds through effective control programmes, the number of human cases will decrease.

2. In 2009 the FSA published results of its UK survey of Campylobacter and Salmonella contamination of fresh chicken at retail sale[2], this survey found the prevalence of Campylobacter in chicken at retail in the UK was 65.2% based on the combining of results from the direct plating and enrichment methods. This survey also highlighted the difficulties in isolating the organism and that the presence/absence method had limitations. Literature suggests that using a combination of presence/absence and enumeration testing provides a more robust measure of Campylobacter prevalence. The overall prevalence figure for the survey was therefore determined by combining the Campylobacter positive results from the 927 samples tested by both presence/absence and enumeration methods.

3. In December 2010 The Food Standards Agency, the UK poultry industry and major retailers agreed a new target that will measure efforts to reduce the levels of Campylobacter in chickens at the end of slaughter. There are three categories of contamination levels and, currently, 19% of birds are in the highest category (>1000 cfu/g)[3]. The target is for the industry to reduce the numbers of these most

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[1] **Scientific Opinion on *Campylobacter* in broiler meat production: control options and performance objectives and/or targets at different stages of the food chain: http://www.efsa.europa.eu/en/efsajournal/doc/2105.pdf**

[2] **Food Standards Agency.2009. UK-wide survey of Campylobacter and Salmonella contamination of fresh chicken at retail sale, Food Standards Agency, London.**
http://www.foodbase.org.uk/admintools/reportdocuments/351-1-676_B18025.pdf

[3] **http://www.food.gov.uk/sites/default/files/full-campy-survey-report.pdf**

contaminated birds in UK poultry houses from 27 to 10 % by the end of December 2015. It is estimated that achievement of this target could mean a reduction in Campylobacter food poisoning of up to 50 %. While this target might not be achieved in the timeframe, a significant decline in the Campylobacter levels on whole chickens towards the end of 2015 is expected.

4. In 2014, a UK-wide survey was established to review the levels of Campylobacter on fresh whole retail chickens and also on their packaging. The intention of the survey was to represent a full 12 month period (mid-February 2014 – mid February 2015) and tested a total of 4,011 samples of whole, UK-produced, fresh chicken. Over 19% of the chickens tested were found to contain Campylobacter at a level above 1000 cfu/g. Just under 73% were positive for Campylobacter at any level (i.e. were found to contain Campylobacter at a level above the detectable limit of 10 cfu/g). Just under 7% of the samples were positive for Campylobacter on the outer packaging (i.e. contained Campylobacter at a level above the detectable limit of 10 cfu/g). For 5 out of the 4,005 samples (for which valid results were available for the outer packaging), the level on the outer packaging was found to be above 1000 cfu/g. While a reduction of the most contaminated chickens to the target level of 10% may not be achieved by the end of December 2015, the evidence from retailer trials show promising results that interventions work.

5. This new survey will extend the Year 1 survey for up to an additional 36 months, including yearly breakpoints, and will investigate the prevalence and levels of Campylobacter contamination in fresh whole chilled chickens and on the outside surface of the packaging at retail using the enumeration method, and will provide valuable information on Campylobacter levels post slaughter. Although the survey will take into consideration seasonal fluctuations in Campylobacter prevalence in retail chickens, this is secondary to the surveys primary function to analyse prevalence among retailers. To draw any definitive conclusions regarding seasonality, we will require data from a number of separate years. The continuation of the retail survey for up to another 36 month additionally intends to identify trends as a result of specific retailer initiatives, such as improvements in biosecurity on farm or processing interventions, which among others, include SonoSteam (a technology combining steam and ultrasound to achieve rapid decontamination of food products such as chicken).

Objectives

A. 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.

B. To determine the prevalence and levels of *Campylobacter* spp. contamination found on the outside packaging of samples collected under Objective 1.

C. To identify *Campylobacter* spp. present and determine susceptibility of an agreed percentage of isolates to a defined range of antimicrobial agents.

D. 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.

Publication of results

6. The survey results will be published under the Code of Practice for Official Statistics. This entails restricted access to raw data and pre-release publication documents.

   At the end of the survey, the results and all the information that has been collected about all of the samples taken for the whole survey period will be published on the Agency's website.

   In addition, sample data and results will be reported within four weeks following the end of a sampling month, and each quarter the Agency will publish an interim report (please see Annex 1 for proposed timeline).

Timetable

7. The survey will consist of a 12 month sampling period plus a further 2 months for data analysis and report preparation. The proposed timetable is available in Annex 1.

SURVEY DESIGN

8. Based on information available, the UK core sample per 12 month period will be 4000 samples. These samples will be taken evenly over a 12-month period unless agreed otherwise with the FSA to address additional objectives.

9. The survey design has been modified to increase statistical confidence in the determination of differences in Campylobacter prevalence among retailers. Therefore the aim is to sample an equal number of chickens (100) from each of the main retailers each quarter. This new design, informed by the knowledge gained during the Year 1 baseline study, increases statistical confidence in the validity of results obtained during the rest of the survey. It also enables to report earlier, more robustly, and with greater confidence, on any improvements which are observed in samples from individual retailers. The market share data will be applied to re-weight the dataset when producing annual estimates of the average prevalence of *Campylobacter* within the UK market.

   Based on more recent market share data (purchased from Kantar in February 2015), the number of retailers to be named has been increased by adding Aldi and Lidl to the list of retailers named in Year 1.

10. The contractor will be responsible for ensuring that the appropriate number of samples is collected in accordance with the sampling plan agreed with the FSA. The number of chickens to be sampled from each UK country will be proportional to retailer market share figures of the respective country. If any deviations are necessary these will be noted in the final report. The contractor will ensure that sampling is evenly distributed throughout the period of the survey and is responsible for selecting and collecting samples at random within these criteria. If possible and in agreement between the contractor and the Agency, a maximum of 4 different chicken types (e.g. different size, brand or rearing) will be collected from any one store on any one occasion; the number of samples collected should be reduced if the sampler is unable to collect 4 different chicken types. A maximum of 2 samples should be taken from butchers and smaller independent stores/grocers at any one time.

11. The aim of this survey is to obtain a total of 4000 samples of whole UK produced raw fresh chilled chicken within any 12 month period. Sample numbers should be reviewed every month to ensure that chickens are being sampled according to the agreed sampling plan.

12. The contractor will provide smaller independent retail outlets with a letter from the Agency informing them that samples have been taken from their premises in order to carry out a survey (Annex 2). For larger retail chains (i.e. Tesco, Asda, Sainsbury's etc.) this is not necessary, as the relevant contact at head office will be sent a list of the premises from which samples have been obtained by the Agency.

SAMPLING

Sample collection

13. It is essential that cross-contamination be avoided during the collection of chicken samples. Precautions will therefore be taken at all stages to ensure that the equipment used during sampling, transport and storage is not contaminated with the pathogens investigated in the survey.

14. Contractors will aim to collect samples at random from the refrigerator cabinet and not necessarily from the front of the display. The surface temperature of the chicken should be recorded using an appropriately calibrated Infra-red thermometer, as should information on whether it was displayed in a temperature-controlled environment e.g. chillers and the overall condition of shelving e.g. was there any visible meat juices on the shelf.

    Only packaged whole fresh chilled UK produced birds should be purchased. Unwrapped chickens may be bought but it should be noted on the sampling form and if available, with an indication of the Use-by Date. Samples, which are packaged, must not show evidence of damage. Each sample then should be placed in a separate sampling bag to avoid the risk of cross-contamination during transport and until testing can take place. For chickens collected from retail premises, the sample should not be purchased if the label on the chicken is not clear, does not include the approval number of the slaughterhouse, or is damaged. Chickens from butchers without labels may be sampled only if approval code can be obtained and must be noted down.

15. **Only unseasoned, fresh whole UK produced chilled whole chickens** should be sampled.

    The chickens sampled may be labeled such as: Whole fresh chilled pre-packed UK-produced chicken and may include ones named as “Standard”, “Value”, “Ex Large”, “Large”, “Medium”, “Roaster”, “Small”, “Barn-reared”, “Free-range”, “Roast-in-bag” etc.

    Samples NOT included are:

    - **Frozen whole chickens, portions (whether fresh or frozen) including legs, breast, thigh and wing portions.**
    - **Any ready basted, marinated, seasoned, herbed, stuffed or pre-prepared whole birds.**
    - **Cooked chickens.**

- **Processed chicken products including goujons, nuggets etc..**

16. Standard produced chickens will be sampled as well as a smaller number of free range and organic chickens (sampling of free range and organic chickens is structured to reflect their market share as outlined in Annex 3). A range of chicken weights will be sampled and weights should be noted down and logged in a separate column of the sample detail spreadsheet. Each sample should, at the point of sampling, have at least 2 days remaining on its Use-by Date.

17. When chilled un-packaged chicken is purchased from butchers/independents the sampling officer may need to enquire about the country of origin; if the bird is/or may not be UK-produced it should not be included in the survey. The sampler should ask the butcher for the approval number which should be present on the bulk packaging. Only samples where approval code can be obtained are to be included in the survey.

18. Each sample should be placed in a plastic bag, which is then sealed. Contractors will ensure that samples are kept at between 1 to 8 °C (±1°C) during transportation and kept dry and out of direct sunlight. A data logger should be placed (not in contact with or close to the cool pack) with the samples to monitor compliance with these requirements. If cool packs are used, samples shall not come into direct contact with their surfaces. Samples should not be frozen. Internal air temperature of the temperature controlled unit and package integrity shall be recorded on receipt at the laboratory.

19. It is essential to identify the approval number (used to be known as the health mark) from each sample so that the origin of the chicken can be determined retrospectively.

Sample information

20. All relevant information available from the sample should be recorded on the sample submission form (Annex 4). As far as possible this information should include the name (please ensure consistency throughout all laboratories) and postcode of the retailer, date and time of purchase, the approval number, weight (in a separate column), use-by date, price, product name, packaging information and display temperature. The sampling sheet is completed with the addition of the results from the microbiological testing. This data are then entered onto a spreadsheet compatible with Microsoft Excel (please see Annex 5 for model spreadsheet with required information).

21. Sampling and results should be reviewed every month to ensure that the chickens sampled could generate statistically valid/meaningful results. The samplers should co-ordinate their sampling with the testing laboratory, project manager and the Agency.

TESTING

Receipt of samples

22. On receipt of the samples, laboratories will check the information recorded by the sampler and complete the relevant sections of the laboratory sample submission form. The information will be entered into the Laboratory Information Management System and transferred from there into a spreadsheet compatible with Microsoft Excel or entered directly onto an Excel sheet. Following examination, the product label itself will be removed and stored if intact and readable.

23. Product information will be captured with digital photographs of each chicken in its packaging and the file will be stored and labelled with the appropriate sample number. Photos or scans are to be stored on suitable digital media under the appropriate sample number separated by sampling months. This will be shared with the Agency via Dropbox or other resources. The scan/photograph will be of a high resolution so that all the relevant labelling details are clear. Following examination, the product label itself shall be removed, cleaned and stored if intact and readable.

24. Chickens sampled should reach the laboratory within 24 hours of sampling. In exceptional situations (e.g. long journeys from the Northern Scottish Isles) this period may be extended to within 48 hours; if the transport period was 48 hours from sampling, the sampler must instruct the laboratory to test on receipt. All samples should always be tested before/on their use-by dates.

Examination

25. Samples of chicken will be examined to ensure that the packaging is intact before testing. If packaging has been damaged during transportation this should be noted on the sampling form before testing. The temperature of the samples will also be recorded on receipt. Satisfactory samples will consider the integrity of packaging as well as sample temperature on receipt and only samples deemed satisfactory on receipt will be considered eligible for testing. Satisfactory sample

receipt may be assigned if samples are within the temperature range of 1 to 8°C (±1°C). If the temperature data logger records temperatures below 1 °C at receipt, the temperature of the sample itself would be measured and if this temperature was below 1 °C the sample would only be assigned as satisfactory if the sample temperature was below 1 °C (±1°C) when collected. Similarly, if the temperature data logger records temperatures above 8 °C, the sample itself would be measured and only deemed acceptable if the temperature at receipt is equal to or lower (±1°C) than the temperature when sampled. Sample receipt procedures would also take into account temperature probe uncertainty and transport time. All samples will be delivered before their use-by date.

26. It is essential that handlers take care to avoid cross contamination between samples and between the chicken and its packaging as well as from the surrounding environment at all stages. Gloves must be worn and changed between each sample of chicken. The work-surface of the bench must be sanitised before unwrapping each chicken. Thorough cleaning of equipment and work surfaces will be undertaken regularly. There must be environmental sampling of the laboratory for test bacteria (Campylobacter) during the testing period at regular intervals. The contractor will carry out examinations in areas dedicated to the examination of survey samples and clearly separated from other potentially contaminated materials.

Methodology

27. The microbiological methodology for the testing of each sample (chicken and packaging) for Campylobacter is as follows:

    The quantitative analysis of Campylobacter in a chicken sample will be based on the method described in **EN/ISO/TS 10272-2:2006** 'Microbiology of food and animal feeding stuffs – Horizontal method for detection and enumeration of *Campylobacter* spp Part 2: Colony-count technique' (please see Annex 6 for detailed method).

Data handling and reporting

28. Within four weeks after each sampling month concludes, the contractor will submit to the Agency a progress report that provides details of the samples taken and the Campylobacter counts. The data for antimicrobial resistance profiles for strains isolated will be submitted independently in agreement with the Agency.

29. The contractor is responsible for collating all results and submitting a final report to the Agency. The report will present summary statistics on the prevalence of Campylobacter, together with a breakdown of the species where appropriate. The results should be subjected to detailed statistical analysis by the contractor; these analyses will be agreed with the Agency's Statistics team prior to commencement.

30. All forms, documentation and electronic files must be retained by the contractor until further notice from the Agency in case of issues arising after the completion of the survey. These should be retained for at least 12 month after completion of the current survey. It is not necessary to provide the Agency with hard copies of forms. However, this information must be readily available to the Agency if required.

31. Every month, the sampling numbers will be assessed to ensure that representative samples are being tested to obtain statistically valid/meaningful results. The contractor is responsible for adjusting the sampling plan every month according to any deviations occurring in the previous month(s) while the FSA is responsible for instructing the contractor on any major changes to the sampling strategy e.g. changes in market-share predictions.

Quality Assurance

32. In order to ensure a high level of accuracy in data entry, data checking and backup, the contractor has to be accredited to the relevant ISO methods by an appropriate organisation (e.g. UKAS). The EN/ISO/TS 10272-2 method is currently being revised to become a full standard and any proposed changes in the final draft may be incorporated providing they are within the scope of the accreditation. The contractor must also be able demonstrate satisfactory performance in the testing of food for *Campylobacter* spp. through participation in an independent proficiency testing scheme. The measurement of uncertainty for enumeration of *Campylobacter* spp. must also be determined and the FSA will visit the contractors during the course of the survey to assess how the work is being carried out.

ANNEX 1: PROPOSED TIMETABLE FOR FIRST YEAR OF SURVEY

June 2015	Protocol finalised
June 2015	MoU signed
Quarter 1 (sampling July/ August/ September)	
Beginning July 2015***** *****covering the quarterly sampling months July/ August/ September	Sampling starts
31 August 2015	July sampling data and results to be submitted to Agency
30 September 2015	August sampling data and results to be submitted to Agency
15 October 2015	Agency to receive spreadsheet containing all **sampling** details for Quarter 1
4 November 2015	September results to be updated and submitted to Agency
26 November 2015	Anticipated Quarter 1 publication
Quarter 2 (sampling October/ November/ December)	
30 November 2015	October sampling data and results to be submitted to Agency
4 January 2016	November sampling data and results to be submitted to Agency
18 January 2016	Agency to receive spreadsheet containing all **sampling** details for Quarter 2
3 February 2016	December results to be updated submitted to Agency
25 February 2016	Anticipated Quarter 2 publication
Quarter 3 (sampling January/ February/ March)	
29 February 2016	January sampling data and results to be submitted to Agency
28 March 2016	February sampling data and results to be submitted to Agency
18 April 2016	Agency to receive spreadsheet containing all **sampling** details for Quarter 3
5 May 2016	March results to be updated submitted to Agency
26 May 2016	Anticipated Quarter 3 publication

Updated 07.07.2015

Quarter 4 (sampling April/ May/ June)	
30 May 2016	April sampling data and results to be submitted to Agency
30 June 2016	May sampling data and results to be submitted to Agency
16 July 2016	Agency to receive spreadsheet containing all **sampling** details for Quarter 4
3 August 2016	June results to be updated and submitted to Agency
25 August 2016	Anticipated Quarter 4 publication
1 July 2016	End of sampling period / annual breakpoint
5 September 2016	Final draft report received
29 September 2016	Final report signed off

ANNEX 2: LETTER TO RETAILERS

Letter to be sent to Retailers during Sampling

Insert Council **Logo &/or Name**

<Date>

Dear

This letter has been given to you by an Environmental Health Officer (EHO)/ Sampling Officer (SO) from [insert name of Contractor].

The EHO / SO is authorised by the [Contractor] to carry out food sampling work, and has purchased chicken from your premises as a food sample, which is to be used for a food surveillance survey.

The aim of this particular survey is to ascertain the incidence and contamination level of Campylobacter in raw UK produced chicken available to consumers at retail in the UK. Whole chickens are being sampled and tested during a 12 month period.

This survey is being funded by the Food Standards Agency which has commissioned [name of Contractor] to carry out the sampling.

Your premise has been visited as one of the retail outlets where people may buy raw chicken - the subject of this survey. The raw chicken purchased from your premises will be taken to [insert name of lab] for testing, and you will be provided with the results of this testing by a letter from the Food Standards Agency. Please note that the survey is not for enforcement purposes.

The results of the samples taken in this survey will be collated and will form part of a report on the incidence and contamination level of the pathogen *Campylobacter* spp. on the surface of the packaging and in fresh whole chilled UK chicken on retail sale within the UK. This report will be published by the Food Standards Agency. At the end of the survey, in line with Food Standards Agency policy on openness and transparency in relation to food safety and matters of interest to consumers relative to food, individual retailers/producers of the chicken sampled will be published on the Agency's website www.food.gov.uk as part of this report.

Should you have any queries, please contact Dr Bettina Mavrommatis, Foodborne Disease Control, Food Safety Policy on the following telephone number:
020 7276 8045 or send an E-mail to
Bettina.Mavrommatis@foodstandards.gsi.gov.uk

Yours sincerely

Updated 07.07.2015

ANNEX 3: SAMPLING PLAN

A UK core sample size of approximately 4000 samples of fresh whole UK produced chilled chicken are needed to achieve the precision required. The sample numbers should be reviewed periodically to ensure that statistically meaningful analyses can be carried out.
The sampling will aim to take place evenly over a 12-month period. The sampling plan is structured to reflect market share data sourced from Kantar (February 2015). Sampling will be kept under review and can, as agreed with the FSA, be revised to accommodate any further survey objectives e.g. over-sample during certain periods.

Table 1 Numbers of chickens to be sampled throughout the UK over 12 month

	Total number of chickens
Total UK	4000
England	3250
Scotland	288
Wales	396
Northern Ireland	66

ENGLAND

Retailer groups	Total no. of chickens	No. of Free-range chickens (of total)	No. of Organic chickens (of total)
Tesco	314	30	1
Asda	321	40	1
Sainsbury's	340	28	5
The Co-operative	313	25	0
Morrisons	333	33	1
Waitrose	369	56	12
M&S	310	2	1
Aldi	313	43	0
Lidl	284	13	0
Butchers	186	*	*
Others1	167	66	0

[1]E.g Iceland, Budgens, Costcutter, Nisa, independents, farm shops, markets stalls.
* samples from butchers can be collected from either category (Standard/ Free Range/ Organic) depending on availability

Updated 07.07.2015

WALES

Retailer groups	Total no. of chickens	No. of Free-range chickens (of total)	No. of Organic chickens (of total)
Tesco	49	4	0
Asda	41	3	0
Sainsbury's	32	3	1
The Co-operative	38	4	0
Morrisons	33	3	0
Waitrose	25	6	0
M&S	34	3	1
Aldi	50	6	0
Lidl	66	2	0
Butchers	10	*	*
Others[1]	18	3	0

[1]E.g Iceland, Budgens, Costcutter, Nisa, independents, farm shops, markets stalls.

* samples from butchers can be collected from either category (Standard/ Free Range/ Organic) depending on availability

SCOTLAND

Retailer groups	Total no. of chickens	No. of Free-range chickens (of total)	No. of Organic chickens (of total)
Tesco	26	5	0
Asda	25	3	0
Sainsbury's	13	1	0
The Co-operative	49	5	0
Morrisons	34	4	0
Waitrose	6	1	0
M&S	56	4	0
Aldi	37	3	0
Lidl	29	2	0
Butchers	4	*	*
Others[1]	9	2	

[1]E.g Iceland, Budgens, Costcutter, Nisa, independents, farm shops, markets stalls.

* samples from butchers can be collected from either category (Standard/ Free Range/ Organic) depending on availability

Updated 07.07.2015

NORTHERN IRELAND

Retailer groups	Total no. of chickens	No. of Free-range chickens (of total)	No. of Organic chickens (of total)
Tesco	11	1	0
Asda	13	2	0
Sainsbury's	15	1	0
The Co-operative	0	0	0
Morrisons	0	0	0
Waitrose	0	0	0
M&S	0	1	0
Aldi	0	0	0
Lidl	21	1	0
Butchers and Others*	6	2	0

*E.g Dunnes, Iceland, Budgens, Costcutter, Nisa, independents, farm shops, markets stalls.

ANNEX 4: SAMPLING FORM

Public Health England

FOOD WATER AND ENVIRONMENTAL MICROBIOLOGY LABORATORY, xxxxxx
xxxxxxxxxxxxxxxxxxxxxxxx
xxxxxxxxxxxxxxx
a UKAS accredited Testing Laboratory No.xxxx
Tel: 0xxxxxxxxx
E-mail: LabFwexxxxx@phe.gov.uk

FSA Campylobacter in chickens 2015-2016

RETAILER: (circle which)	ALDI / ASDA / CO-OP / LIDL / M&S MORRISONS / SAINSBURYS TESCO / WAITROSE / BUTCHERS* OTHER*
Post Code:	.....
*If BUTCHERS or OTHER give premises name and address:	....

AFFIX LABORATORY NUMBER HERE

Sampling done by (tick): PHE lab ☐ MH Scientific ☐
CRCE ☐ N Ireland lab ☐
Sender contact tel # .....
Cool Box ID: .....

Sample collected by: ..... Date collected: ..... Time collected (24 hour clock): ..... Temperature at collection .....°C

Sample Details
Sample description (enter full name of product as it appears on the label)
.....
Use by date: dd/mm/yy...../...../..... Batch Number (Approval Number .....)

Additional sample details: Enter answers to the questions and **TICK ALL BOXES THAT APPLY**

Q#	Question	Response
Q1	Display unit	Dry ☐ Wet ☐ Dirty and / or bloody ☐
Q2	Weight of product as shown on packaging	.....Grams
Q3	Price	£.....
Q4	Type of chicken (as shown on packaging)	Standard ☐ Free range ☐ Organic ☐ Choose Standard if type not stated
Q5	Other chicken details	Halal ☐ Corn fed ☐ Other ☐ (give details): .....
Q6	Assurance scheme	Red Tractor ☐ Other ☐ (give details e.g. Freedom Foods): .....
Q7	Packaging details	Wrapped tight ☐ Tray present ☐ Modified atmosphere ☐ Other (give details): .....
Q8	Visible liquid in pack?	Yes ☐ No ☐
Q9	Other sampling comments:	
Q10	**For Lab Use Only**	Weight of neck skin in sample .....Grams

LABORATORY USE ONLY (Record details of unsatisfactory findings in comments)

Date received: ...../...../.....
Time received: .....
Received by: .....
Received from: .....

Data logger / probe ID: .....
Air / In between pack (delete as appropriate)
Temp. on receipt: .....°C

Samples & Receipt: ☐ SATIS ☐ UNSATIS

Comments:

Associate with STARLIMS Study No.:

FSA Campy in chickens 2015 submittal form v1

ANNEX 5: Model data submission template (as Excel)

Country	NI lab ref	Post code	Sample Number	Temp at Collection (Store) °C	Temp on Receipt (Lab) °C	Date Examined	Retailer store	Retailer details (if Others/ Butchers)	Premises Address (Others/ butcher)	Sample Details	chicken weight (g)	Other Packaging Details	Other packaging comments	Chicken type	Specify other production types	Cost of chicken (£)	Approval Number	Use by date	breastskin taken for analysis (g)	Test Name	Result	Units	Speciation	Result
as before	*as before*	*as before*	*as before*	*please specify accurate (without characters)*	*please specify accurate (without characters)*	*consistent date format*	*consistency throughout*			*please specify here*	*number in gram without characters*	*please specify here*	*please specify here*	*specify consistently*	*specify if described by following*	*specify (number only; no characters)*	*specify (number only; no characters)*	*consistent date format*	*please specify breastskin taken for analysis (g)*					
				5	6.2	12/08/2015	Co-op			Small whole chicken Fresh Class A	4269	Roast-in-bag	liquid visible in pack	STD	Halal	2.99	8005	12/08/2015						
							Asda					No tray, modified atmosphere packed		FR	cornfed									
							Morrisons					Tray present, modified atmosphere packed.		O										
							Sainsbury's					No tray. Plastic, lightly wrapped.												
							Tesco					Loose bag. No tray.												
							Lidl																	
							Waitrose																	
							Aldi																	
							M&S																	
							Others	NISA																
								Iceland																
								etc.																
							Butchers	Pat The Butcher	1 Little Aston Lane, Little Aston, Birmingham															

ANNEX 6: LABORATORY METHODOLOGY

Overview

Chicken neck-skin samples and the outside surface of packaging will be analysed for Campylobacter. Wear suitable single-use gloves for handling the packaged chicken, changing gloves after each sample.

Outer packaging swab

Place the wrapped chicken, with the outer bag folded away from the pack label onto a clean surface and take a picture (with sample number and pack label clearly visible) and retain label after examination.
Add 10 ml of Maximum Recovery Diluent (MRD) to a SpongeSicle™swab and ensure the swab is thoroughly wetted.
Remove the outer sample bag and place the wrapped chicken on a previously disinfected dry plastic tray wearing disposable gloves.
Swab the entire outer surface of the chicken packaging using aseptic technique (swab whole pack twice using both side of the swab). In case of 'Roast/ Cook in bag' chicken, which for some retailers can come double-bagged, swab the outer bag for enumeration.
Replace the swab in its bag breaking off the stick and then stomaching the swab for 30 s.
Using a sterile pastette remove > 2 ml into a sterile container for enumeration as described below.

Chicken skin

Wearing a fresh pair of disposable gloves, remove the chicken from its wrapping, taking care not to allow contact between the chicken and outer packaging. Using sterile instruments (e.g. scissors and tweezers) aseptically remove skin from the neck area (if < 25 g neck-skin is available top up with breast skin (**record weight of this**)) to make a 25 g test portion, avoiding fat and place this into a sterile bag.
Add 225 ml BPW and homogenise for one minute. Remove > 3 ml for enumeration as described below.

Enumeration of Campylobacter spp.

Enumerate *Campylobacter* spp. by the surface plate method as described in the PHE Methods - Detection and enumeration of *Campylobacter* spp.: *FNES15 (*F21) v2 (see Appendix I). This method is based ISO/TS 10272-2:2006 Microbiology of food and animal feeding stuffs -- Horizontal method for detection and enumeration of Campylobacter spp. -- Part 2: Colony-count technique and entails the following:

Plating of 1 ml of the packaging swab liquid onto three modified cefoperazone, charcoal deoxycholate agars (CCDA plates: e.g. Oxoid CM739 with Oxoid selective supplement SR155).

Plating of 1 ml of the chicken skin homogenate onto three CCDA plates and 100 µl onto duplicate CCDA plates. Prepare two further 10-fold dilutions in MRD and plate 100 µl of each of these in duplicate onto CCDA plates.

Incubate CCDA plates in a microaerophilic atmosphere at 41.5 ± 1°C for 44 ± 4 h.

Counting and confirmation of suspect/typical colonies

Count plates from those with less than 150 colonies, where possible. As the bacteria rapidly deteriorate in air progress confirmation of colonies immediately. Pick 5 (or less if less present) colonies (based on typical colony morphology) and sub-culture onto Columbia Blood Agar (containing 5 % (v/v) defibrinated blood). Check that growth is

absent after incubation under aerobic conditions after 48 h and check for typical growth in a microaerophilic atmosphere at 41.5 ºC. Confirm oxidase reaction of pure cultures and typical Campylobacter cell morphology (small, slim, curved or spiral, Gram-negative rods/motility (wet mount/phase contrast)). Commercially available latex agglutination test kits can be used to identify campylobacters (e.g. Microscreen® campylobacter (Microgen bioproducts) and Dryspot campylobacter test (Oxoid Ltd) consistent with local Standard Operating Procedures.

Isolates of *Campylobacter* spp. will be sent, as soon as possible, to the Gastrointestinal Bacteria Reference Unit (GBRU) CampyLab, PHE London (Hays DX number DX653008) for speciation and antibiotic resistance testing. One isolate from each positive sample will be sent and archived by GBRU. Isolates sent to GBRU must be clearly labelled with their sample number and the name of the referring laboratory.

A UK WIDE MICROBIOLOGICAL SURVEY OF CAMPYLOBACTER CONTAMINATION IN FRESH WHOLE CHILLED CHICKENS AT RETAIL SALE (Year 2/ 3/ 4)

PROTOCOL

Revised July 2015

Updated 07.07.2015

CONTENTS

ABBREVIATIONS

CCDA-	Charcoal Cefoperazone Deoxycholate Agar
DH -	Department of Health
FSA -	Food Standards Agency
h -	Hour(s)
PHE -	Public Health England
ISO-	International Standards Organisation
LGP-	Laboratory of Gastrointestinal Pathogens
mL -	Millilitres
mm -	Millimetres
s -	Seconds
MS-	Microbiological Services
UKAS-	United Kingdom Accreditation Service

OUTLINE

Background

1. The Food Standards Agency has a key role in preventing foodborne illnesses. The Strategic Plan aims to reduce foodborne disease further and has set a target to reduce Campylobacter contamination in raw chicken.

   Campylobacter is the most prominent bacterium associated with foodborne disease within the United Kingdom. Foodborne Campylobacter is estimated to make more than 280,000 people ill each year in the UK and is the biggest cause of food poisoning. An EFSA Opinion[1] stated that up to 80% of cases can be attributed to raw poultry meat. It is hoped that by reducing the number of highly positive birds through effective control programmes, the number of human cases will decrease.

2. In 2009 the FSA published results of its UK survey of Campylobacter and Salmonella contamination of fresh chicken at retail sale[2], this survey found the prevalence of Campylobacter in chicken at retail in the UK was 65.2% based on the combining of results from the direct plating and enrichment methods. This survey also highlighted the difficulties in isolating the organism and that the presence/absence method had limitations. Literature suggests that using a combination of presence/absence and enumeration testing provides a more robust measure of Campylobacter prevalence. The overall prevalence figure for the survey was therefore determined by combining the Campylobacter positive results from the 927 samples tested by both presence/absence and enumeration methods.

3. In December 2010 The Food Standards Agency, the UK poultry industry and major retailers agreed a new target that will measure efforts to reduce the levels of Campylobacter in chickens at the end of slaughter. There are three categories of contamination levels and, currently, 19% of birds are in the highest category (>1000 cfu/g)[3]. The target is for the industry to reduce the numbers of these most

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[1] **Scientific Opinion on *Campylobacter* in broiler meat production: control options and performance objectives and/or targets at different stages of the food chain: http://www.efsa.europa.eu/en/efsajournal/doc/2105.pdf**

[2] **Food Standards Agency.2009. UK-wide survey of Campylobacter and Salmonella contamination of fresh chicken at retail sale, Food Standards Agency, London.**
http://www.foodbase.org.uk/admintools/reportdocuments/351-1-676_B18025.pdf

[3] **http://www.food.gov.uk/sites/default/files/full-campy-survey-report.pdf**

contaminated birds in UK poultry houses from 27 to 10 % by the end of December 2015. It is estimated that achievement of this target could mean a reduction in Campylobacter food poisoning of up to 50 %. While this target might not be achieved in the timeframe, a significant decline in the Campylobacter levels on whole chickens towards the end of 2015 is expected.

4. In 2014, a UK-wide survey was established to review the levels of Campylobacter on fresh whole retail chickens and also on their packaging. The intention of the survey was to represent a full 12 month period (mid-February 2014 – mid February 2015) and tested a total of 4,011 samples of whole, UK-produced, fresh chicken. Over 19% of the chickens tested were found to contain Campylobacter at a level above 1000 cfu/g. Just under 73% were positive for Campylobacter at any level (i.e. were found to contain Campylobacter at a level above the detectable limit of 10 cfu/g). Just under 7% of the samples were positive for Campylobacter on the outer packaging (i.e. contained Campylobacter at a level above the detectable limit of 10 cfu/g). For 5 out of the 4,005 samples (for which valid results were available for the outer packaging), the level on the outer packaging was found to be above 1000 cfu/g. While a reduction of the most contaminated chickens to the target level of 10% may not be achieved by the end of December 2015, the evidence from retailer trials show promising results that interventions work.

5. This new survey will extend the Year 1 survey for up to an additional 36 months, including yearly breakpoints, and will investigate the prevalence and levels of Campylobacter contamination in fresh whole chilled chickens and on the outside surface of the packaging at retail using the enumeration method, and will provide valuable information on Campylobacter levels post slaughter. Although the survey will take into consideration seasonal fluctuations in Campylobacter prevalence in retail chickens, this is secondary to the surveys primary function to analyse prevalence among retailers. To draw any definitive conclusions regarding seasonality, we will require data from a number of separate years. The continuation of the retail survey for up to another 36 month additionally intends to identify trends as a result of specific retailer initiatives, such as improvements in biosecurity on farm or processing interventions, which among others, include SonoSteam (a technology combining steam and ultrasound to achieve rapid decontamination of food products such as chicken).

Objectives

A. 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.

B. To determine the prevalence and levels of *Campylobacter* spp. contamination found on the outside packaging of samples collected under Objective 1.

C. To identify *Campylobacter* spp. present and determine susceptibility of an agreed percentage of isolates to a defined range of antimicrobial agents.

D. 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.

Publication of results

6. The survey results will be published under the Code of Practice for Official Statistics. This entails restricted access to raw data and pre-release publication documents.

   At the end of the survey, the results and all the information that has been collected about all of the samples taken for the whole survey period will be published on the Agency's website.

   In addition, sample data and results will be reported within four weeks following the end of a sampling month, and each quarter the Agency will publish an interim report (please see Annex 1 for proposed timeline).

Timetable

7. The survey will consist of a 12 month sampling period plus a further 2 months for data analysis and report preparation. The proposed timetable is available in Annex 1.

SURVEY DESIGN

8. Based on information available, the UK core sample per 12 month period will be 4000 samples. These samples will be taken evenly over a 12-month period unless agreed otherwise with the FSA to address additional objectives.

9. The survey design has been modified to increase statistical confidence in the determination of differences in Campylobacter prevalence among retailers. Therefore the aim is to sample an equal number of chickens (100) from each of the main retailers each quarter. This new design, informed by the knowledge gained during the Year 1 baseline study, increases statistical confidence in the validity of results obtained during the rest of the survey. It also enables to report earlier, more robustly, and with greater confidence, on any improvements which are observed in samples from individual retailers. The market share data will be applied to re-weight the dataset when producing annual estimates of the average prevalence of *Campylobacter* within the UK market.

   Based on more recent market share data (purchased from Kantar in February 2015), the number of retailers to be named has been increased by adding Aldi and Lidl to the list of retailers named in Year 1.

10. The contractor will be responsible for ensuring that the appropriate number of samples is collected in accordance with the sampling plan agreed with the FSA. The number of chickens to be sampled from each UK country will be proportional to retailer market share figures of the respective country. If any deviations are necessary these will be noted in the final report. The contractor will ensure that sampling is evenly distributed throughout the period of the survey and is responsible for selecting and collecting samples at random within these criteria. If possible and in agreement between the contractor and the Agency, a maximum of 4 different chicken types (e.g. different size, brand or rearing) will be collected from any one store on any one occasion; the number of samples collected should be reduced if the sampler is unable to collect 4 different chicken types. A maximum of 2 samples should be taken from butchers and smaller independent stores/grocers at any one time.

11. The aim of this survey is to obtain a total of 4000 samples of whole UK produced raw fresh chilled chicken within any 12 month period. Sample numbers should be reviewed every month to ensure that chickens are being sampled according to the agreed sampling plan.

12. The contractor will provide smaller independent retail outlets with a letter from the Agency informing them that samples have been taken from their premises in order to carry out a survey (Annex 2). For larger retail chains (i.e. Tesco, Asda, Sainsbury's etc.) this is not necessary, as the relevant contact at head office will be sent a list of the premises from which samples have been obtained by the Agency.

SAMPLING

Sample collection

13. It is essential that cross-contamination be avoided during the collection of chicken samples. Precautions will therefore be taken at all stages to ensure that the equipment used during sampling, transport and storage is not contaminated with the pathogens investigated in the survey.

14. Contractors will aim to collect samples at random from the refrigerator cabinet and not necessarily from the front of the display. The surface temperature of the chicken should be recorded using an appropriately calibrated Infra-red thermometer, as should information on whether it was displayed in a temperature-controlled environment e.g. chillers and the overall condition of shelving e.g. was there any visible meat juices on the shelf.

    Only packaged whole fresh chilled UK produced birds should be purchased. Unwrapped chickens may be bought but it should be noted on the sampling form and if available, with an indication of the Use-by Date. Samples, which are packaged, must not show evidence of damage. Each sample then should be placed in a separate sampling bag to avoid the risk of cross-contamination during transport and until testing can take place. For chickens collected from retail premises, the sample should not be purchased if the label on the chicken is not clear, does not include the approval number of the slaughterhouse, or is damaged. Chickens from butchers without labels may be sampled only if approval code can be obtained and must be noted down.

15. **Only unseasoned, fresh whole UK produced chilled whole chickens** should be sampled.

    The chickens sampled may be labeled such as: Whole fresh chilled pre-packed UK-produced chicken and may include ones named as “Standard”, “Value”, “Ex Large”, “Large”, “Medium”, “Roaster”, “Small”, “Barn-reared”, “Free-range”, “Roast-in-bag” etc.

    Samples NOT included are:

    - **Frozen whole chickens, portions (whether fresh or frozen) including legs, breast, thigh and wing portions.**
    - **Any ready basted, marinated, seasoned, herbed, stuffed or pre-prepared whole birds.**
    - **Cooked chickens.**

- **Processed chicken products including goujons, nuggets etc..**

16. Standard produced chickens will be sampled as well as a smaller number of free range and organic chickens (sampling of free range and organic chickens is structured to reflect their market share as outlined in Annex 3). A range of chicken weights will be sampled and weights should be noted down and logged in a separate column of the sample detail spreadsheet. Each sample should, at the point of sampling, have at least 2 days remaining on its Use-by Date.

17. When chilled un-packaged chicken is purchased from butchers/independents the sampling officer may need to enquire about the country of origin; if the bird is/or may not be UK-produced it should not be included in the survey. The sampler should ask the butcher for the approval number which should be present on the bulk packaging. Only samples where approval code can be obtained are to be included in the survey.

18. Each sample should be placed in a plastic bag, which is then sealed. Contractors will ensure that samples are kept at between 1 to 8 °C (±1°C) during transportation and kept dry and out of direct sunlight. A data logger should be placed (not in contact with or close to the cool pack) with the samples to monitor compliance with these requirements. If cool packs are used, samples shall not come into direct contact with their surfaces. Samples should not be frozen. Internal air temperature of the temperature controlled unit and package integrity shall be recorded on receipt at the laboratory.

19. It is essential to identify the approval number (used to be known as the health mark) from each sample so that the origin of the chicken can be determined retrospectively.

Sample information

20. All relevant information available from the sample should be recorded on the sample submission form (Annex 4). As far as possible this information should include the name (please ensure consistency throughout all laboratories) and postcode of the retailer, date and time of purchase, the approval number, weight (in a separate column), use-by date, price, product name, packaging information and display temperature. The sampling sheet is completed with the addition of the results from the microbiological testing. This data are then entered onto a spreadsheet compatible with Microsoft Excel (please see Annex 5 for model spreadsheet with required information).

21. Sampling and results should be reviewed every month to ensure that the chickens sampled could generate statistically valid/meaningful results. The samplers should co-ordinate their sampling with the testing laboratory, project manager and the Agency.

TESTING

Receipt of samples

22. On receipt of the samples, laboratories will check the information recorded by the sampler and complete the relevant sections of the laboratory sample submission form. The information will be entered into the Laboratory Information Management System and transferred from there into a spreadsheet compatible with Microsoft Excel or entered directly onto an Excel sheet. Following examination, the product label itself will be removed and stored if intact and readable.

23. Product information will be captured with digital photographs of each chicken in its packaging and the file will be stored and labelled with the appropriate sample number. Photos or scans are to be stored on suitable digital media under the appropriate sample number separated by sampling months. This will be shared with the Agency via Dropbox or other resources. The scan/photograph will be of a high resolution so that all the relevant labelling details are clear. Following examination, the product label itself shall be removed, cleaned and stored if intact and readable.

24. Chickens sampled should reach the laboratory within 24 hours of sampling. In exceptional situations (e.g. long journeys from the Northern Scottish Isles) this period may be extended to within 48 hours; if the transport period was 48 hours from sampling, the sampler must instruct the laboratory to test on receipt. All samples should always be tested before/on their use-by dates.

Examination

25. Samples of chicken will be examined to ensure that the packaging is intact before testing. If packaging has been damaged during transportation this should be noted on the sampling form before testing. The temperature of the samples will also be recorded on receipt. Satisfactory samples will consider the integrity of packaging as well as sample temperature on receipt and only samples deemed satisfactory on receipt will be considered eligible for testing. Satisfactory sample

receipt may be assigned if samples are within the temperature range of 1 to 8°C (±1°C). If the temperature data logger records temperatures below 1 °C at receipt, the temperature of the sample itself would be measured and if this temperature was below 1 °C the sample would only be assigned as satisfactory if the sample temperature was below 1 °C (±1°C) when collected. Similarly, if the temperature data logger records temperatures above 8 °C, the sample itself would be measured and only deemed acceptable if the temperature at receipt is equal to or lower (±1°C) than the temperature when sampled. Sample receipt procedures would also take into account temperature probe uncertainty and transport time. All samples will be delivered before their use-by date.

26. It is essential that handlers take care to avoid cross contamination between samples and between the chicken and its packaging as well as from the surrounding environment at all stages. Gloves must be worn and changed between each sample of chicken. The work-surface of the bench must be sanitised before unwrapping each chicken. Thorough cleaning of equipment and work surfaces will be undertaken regularly. There must be environmental sampling of the laboratory for test bacteria (Campylobacter) during the testing period at regular intervals. The contractor will carry out examinations in areas dedicated to the examination of survey samples and clearly separated from other potentially contaminated materials.

Methodology

27. The microbiological methodology for the testing of each sample (chicken and packaging) for Campylobacter is as follows:

    The quantitative analysis of Campylobacter in a chicken sample will be based on the method described in **EN/ISO/TS 10272-2:2006** 'Microbiology of food and animal feeding stuffs – Horizontal method for detection and enumeration of *Campylobacter* spp Part 2: Colony-count technique' (please see Annex 6 for detailed method).

Data handling and reporting

28. Within four weeks after each sampling month concludes, the contractor will submit to the Agency a progress report that provides details of the samples taken and the Campylobacter counts. The data for antimicrobial resistance profiles for strains isolated will be submitted independently in agreement with the Agency.

29. The contractor is responsible for collating all results and submitting a final report to the Agency. The report will present summary statistics on the prevalence of Campylobacter, together with a breakdown of the species where appropriate. The results should be subjected to detailed statistical analysis by the contractor; these analyses will be agreed with the Agency's Statistics team prior to commencement.

30. All forms, documentation and electronic files must be retained by the contractor until further notice from the Agency in case of issues arising after the completion of the survey. These should be retained for at least 12 month after completion of the current survey. It is not necessary to provide the Agency with hard copies of forms. However, this information must be readily available to the Agency if required.

31. Every month, the sampling numbers will be assessed to ensure that representative samples are being tested to obtain statistically valid/meaningful results. The contractor is responsible for adjusting the sampling plan every month according to any deviations occurring in the previous month(s) while the FSA is responsible for instructing the contractor on any major changes to the sampling strategy e.g. changes in market-share predictions.

Quality Assurance

32. In order to ensure a high level of accuracy in data entry, data checking and backup, the contractor has to be accredited to the relevant ISO methods by an appropriate organisation (e.g. UKAS). The EN/ISO/TS 10272-2 method is currently being revised to become a full standard and any proposed changes in the final draft may be incorporated providing they are within the scope of the accreditation. The contractor must also be able demonstrate satisfactory performance in the testing of food for *Campylobacter* spp. through participation in an independent proficiency testing scheme. The measurement of uncertainty for enumeration of *Campylobacter* spp. must also be determined and the FSA will visit the contractors during the course of the survey to assess how the work is being carried out.

ANNEX 1: PROPOSED TIMETABLE FOR FIRST YEAR OF SURVEY

June 2015	Protocol finalised
June 2015	MoU signed
Quarter 1 (sampling July/ August/ September)	
Beginning July 2015***** *****covering the quarterly sampling months July/ August/ September	Sampling starts
31 August 2015	July sampling data and results to be submitted to Agency
30 September 2015	August sampling data and results to be submitted to Agency
15 October 2015	Agency to receive spreadsheet containing all **sampling** details for Quarter 1
4 November 2015	September results to be updated and submitted to Agency
26 November 2015	Anticipated Quarter 1 publication
Quarter 2 (sampling October/ November/ December)	
30 November 2015	October sampling data and results to be submitted to Agency
4 January 2016	November sampling data and results to be submitted to Agency
18 January 2016	Agency to receive spreadsheet containing all **sampling** details for Quarter 2
3 February 2016	December results to be updated submitted to Agency
25 February 2016	Anticipated Quarter 2 publication
Quarter 3 (sampling January/ February/ March)	
29 February 2016	January sampling data and results to be submitted to Agency
28 March 2016	February sampling data and results to be submitted to Agency
18 April 2016	Agency to receive spreadsheet containing all **sampling** details for Quarter 3
5 May 2016	March results to be updated submitted to Agency
26 May 2016	Anticipated Quarter 3 publication

Updated 07.07.2015

Quarter 4 (sampling April/ May/ June)	
30 May 2016	April sampling data and results to be submitted to Agency
30 June 2016	May sampling data and results to be submitted to Agency
16 July 2016	Agency to receive spreadsheet containing all **sampling** details for Quarter 4
3 August 2016	June results to be updated and submitted to Agency
25 August 2016	Anticipated Quarter 4 publication
1 July 2016	End of sampling period / annual breakpoint
5 September 2016	Final draft report received
29 September 2016	Final report signed off

ANNEX 2: LETTER TO RETAILERS

Letter to be sent to Retailers during Sampling

Insert Council **Logo &/or Name**

<Date>

Dear

This letter has been given to you by an Environmental Health Officer (EHO)/ Sampling Officer (SO) from [insert name of Contractor].

The EHO / SO is authorised by the [Contractor] to carry out food sampling work, and has purchased chicken from your premises as a food sample, which is to be used for a food surveillance survey.

The aim of this particular survey is to ascertain the incidence and contamination level of Campylobacter in raw UK produced chicken available to consumers at retail in the UK. Whole chickens are being sampled and tested during a 12 month period.

This survey is being funded by the Food Standards Agency which has commissioned [name of Contractor] to carry out the sampling.

Your premise has been visited as one of the retail outlets where people may buy raw chicken - the subject of this survey. The raw chicken purchased from your premises will be taken to [insert name of lab] for testing, and you will be provided with the results of this testing by a letter from the Food Standards Agency. Please note that the survey is not for enforcement purposes.

The results of the samples taken in this survey will be collated and will form part of a report on the incidence and contamination level of the pathogen *Campylobacter* spp. on the surface of the packaging and in fresh whole chilled UK chicken on retail sale within the UK. This report will be published by the Food Standards Agency. At the end of the survey, in line with Food Standards Agency policy on openness and transparency in relation to food safety and matters of interest to consumers relative to food, individual retailers/producers of the chicken sampled will be published on the Agency's website www.food.gov.uk as part of this report.

Should you have any queries, please contact Dr Bettina Mavrommatis, Foodborne Disease Control, Food Safety Policy on the following telephone number:
020 7276 8045 or send an E-mail to
Bettina.Mavrommatis@foodstandards.gsi.gov.uk

Yours sincerely

Updated 07.07.2015

ANNEX 3: SAMPLING PLAN

A UK core sample size of approximately 4000 samples of fresh whole UK produced chilled chicken are needed to achieve the precision required. The sample numbers should be reviewed periodically to ensure that statistically meaningful analyses can be carried out.
The sampling will aim to take place evenly over a 12-month period. The sampling plan is structured to reflect market share data sourced from Kantar (February 2015). Sampling will be kept under review and can, as agreed with the FSA, be revised to accommodate any further survey objectives e.g. over-sample during certain periods.

Table 1 Numbers of chickens to be sampled throughout the UK over 12 month

	Total number of chickens
Total UK	4000
England	3250
Scotland	288
Wales	396
Northern Ireland	66

ENGLAND

Retailer groups	Total no. of chickens	No. of Free-range chickens (of total)	No. of Organic chickens (of total)
Tesco	314	30	1
Asda	321	40	1
Sainsbury's	340	28	5
The Co-operative	313	25	0
Morrisons	333	33	1
Waitrose	369	56	12
M&S	310	2	1
Aldi	313	43	0
Lidl	284	13	0
Butchers	186	*	*
Others1	167	66	0

[1]E.g Iceland, Budgens, Costcutter, Nisa, independents, farm shops, markets stalls.
* samples from butchers can be collected from either category (Standard/ Free Range/ Organic) depending on availability

Updated 07.07.2015

WALES

Retailer groups	Total no. of chickens	No. of Free-range chickens (of total)	No. of Organic chickens (of total)
Tesco	49	4	0
Asda	41	3	0
Sainsbury's	32	3	1
The Co-operative	38	4	0
Morrisons	33	3	0
Waitrose	25	6	0
M&S	34	3	1
Aldi	50	6	0
Lidl	66	2	0
Butchers	10	*	*
Others[1]	18	3	0

[1]E.g Iceland, Budgens, Costcutter, Nisa, independents, farm shops, markets stalls.

* samples from butchers can be collected from either category (Standard/ Free Range/ Organic) depending on availability

SCOTLAND

Retailer groups	Total no. of chickens	No. of Free-range chickens (of total)	No. of Organic chickens (of total)
Tesco	26	5	0
Asda	25	3	0
Sainsbury's	13	1	0
The Co-operative	49	5	0
Morrisons	34	4	0
Waitrose	6	1	0
M&S	56	4	0
Aldi	37	3	0
Lidl	29	2	0
Butchers	4	*	*
Others[1]	9	2	

[1]E.g Iceland, Budgens, Costcutter, Nisa, independents, farm shops, markets stalls.

* samples from butchers can be collected from either category (Standard/ Free Range/ Organic) depending on availability

Updated 07.07.2015

NORTHERN IRELAND

Retailer groups	Total no. of chickens	No. of Free-range chickens (of total)	No. of Organic chickens (of total)
Tesco	11	1	0
Asda	13	2	0
Sainsbury's	15	1	0
The Co-operative	0	0	0
Morrisons	0	0	0
Waitrose	0	0	0
M&S	0	1	0
Aldi	0	0	0
Lidl	21	1	0
Butchers and Others*	6	2	0

*E.g Dunnes, Iceland, Budgens, Costcutter, Nisa, independents, farm shops, markets stalls.

ANNEX 4: SAMPLING FORM

Public Health England

FOOD WATER AND ENVIRONMENTAL MICROBIOLOGY LABORATORY, xxxxxx
xxxxxxxxxxxxxxxxxxxxxxxx
xxxxxxxxxxxxxxx
a UKAS accredited Testing Laboratory No.xxxx
Tel: 0xxxxxxxxx
E-mail: LabFwexxxxx@phe.gov.uk

FSA Campylobacter in chickens 2015-2016

RETAILER: (circle which)	ALDI / ASDA / CO-OP / LIDL / M&S MORRISONS / SAINSBURYS TESCO / WAITROSE / BUTCHERS* OTHER*
Post Code:	.....
*If BUTCHERS or OTHER give premises name and address:	....

AFFIX LABORATORY NUMBER HERE

Sampling done by (tick): PHE lab ☐ MH Scientific ☐
CRCE ☐ N Ireland lab ☐
Sender contact tel # .....
Cool Box ID: .....

Sample collected by: ..... Date collected: ..... Time collected (24 hour clock): ..... Temperature at collection .....°C

Sample Details
Sample description (enter full name of product as it appears on the label)
.....
Use by date: dd/mm/yy...../...../..... Batch Number (Approval Number .....)

Additional sample details: Enter answers to the questions and **TICK ALL BOXES THAT APPLY**

Q#	Question	Response
Q1	Display unit	Dry ☐ Wet ☐ Dirty and / or bloody ☐
Q2	Weight of product as shown on packaging	.....Grams
Q3	Price	£.....
Q4	Type of chicken (as shown on packaging)	Standard ☐ Free range ☐ Organic ☐ Choose Standard if type not stated
Q5	Other chicken details	Halal ☐ Corn fed ☐ Other ☐ (give details): .....
Q6	Assurance scheme	Red Tractor ☐ Other ☐ (give details e.g. Freedom Foods): .....
Q7	Packaging details	Wrapped tight ☐ Tray present ☐ Modified atmosphere ☐ Other (give details): .....
Q8	Visible liquid in pack?	Yes ☐ No ☐
Q9	Other sampling comments:	
Q10	**For Lab Use Only**	Weight of neck skin in sample .....Grams

LABORATORY USE ONLY (Record details of unsatisfactory findings in comments)

Date received: ...../...../.....
Time received: .....
Received by: .....
Received from: .....

Data logger / probe ID: .....
Air / In between pack (delete as appropriate)
Temp. on receipt: .....°C

Samples & Receipt: ☐ SATIS ☐ UNSATIS

Comments:

Associate with STARLIMS Study No.:

FSA Campy in chickens 2015 submittal form v1

ANNEX 5: Model data submission template (as Excel)

Country	NI lab ref	Post code	Sample Number	Temp at Collection (Store) °C	Temp on Receipt (Lab) °C	Date Examined	Retailer store	Retailer details (if Others/ Butchers)	Premises Address (Others/ butcher)	Sample Details	chicken weight (g)	Other Packaging Details	Other packaging comments	Chicken type	Specify other production types	Cost of chicken (£)	Approval Number	Use by date	breastskin taken for analysis (g)	Test Name	Result	Units	Speciation	Result
as before	*as before*	*as before*	*as before*	*please specify accurate (without characters)*	*please specify accurate (without characters)*	*consistent date format*	*consistency throughout*			*please specify here*	*number in gram without characters*	*please specify here*	*please specify here*	*specify consistently*	*specify if described by following*	*specify (number only; no characters)*	*specify (number only; no characters)*	*consistent date format*	*please specify breastskin taken for analysis (g)*					
				5	6.2	12/08/2015	Co-op			Small whole chicken Fresh Class A	4269	Roast-in-bag	liquid visible in pack	STD	Halal	2.99	8005	12/08/2015						
							Asda					No tray, modified atmosphere packed		FR	cornfed									
							Morrisons					Tray present, modified atmosphere packed.		O										
							Sainsbury's					No tray. Plastic, lightly wrapped.												
							Tesco					Loose bag. No tray.												
							Lidl																	
							Waitrose																	
							Aldi																	
							M&S																	
							Others	NISA																
								Iceland																
								etc.																
							Butchers	Pat The Butcher	1 Little Aston Lane, Little Aston, Birmingham															

ANNEX 6: LABORATORY METHODOLOGY

Overview

Chicken neck-skin samples and the outside surface of packaging will be analysed for Campylobacter. Wear suitable single-use gloves for handling the packaged chicken, changing gloves after each sample.

Outer packaging swab

Place the wrapped chicken, with the outer bag folded away from the pack label onto a clean surface and take a picture (with sample number and pack label clearly visible) and retain label after examination.
Add 10 ml of Maximum Recovery Diluent (MRD) to a SpongeSicle™swab and ensure the swab is thoroughly wetted.
Remove the outer sample bag and place the wrapped chicken on a previously disinfected dry plastic tray wearing disposable gloves.
Swab the entire outer surface of the chicken packaging using aseptic technique (swab whole pack twice using both side of the swab). In case of 'Roast/ Cook in bag' chicken, which for some retailers can come double-bagged, swab the outer bag for enumeration.
Replace the swab in its bag breaking off the stick and then stomaching the swab for 30 s.
Using a sterile pastette remove > 2 ml into a sterile container for enumeration as described below.

Chicken skin

Wearing a fresh pair of disposable gloves, remove the chicken from its wrapping, taking care not to allow contact between the chicken and outer packaging. Using sterile instruments (e.g. scissors and tweezers) aseptically remove skin from the neck area (if < 25 g neck-skin is available top up with breast skin (**record weight of this**)) to make a 25 g test portion, avoiding fat and place this into a sterile bag.
Add 225 ml BPW and homogenise for one minute. Remove > 3 ml for enumeration as described below.

Enumeration of Campylobacter spp.

Enumerate *Campylobacter* spp. by the surface plate method as described in the PHE Methods - Detection and enumeration of *Campylobacter* spp.: *FNES15 (*F21) v2 (see Appendix I). This method is based ISO/TS 10272-2:2006 Microbiology of food and animal feeding stuffs -- Horizontal method for detection and enumeration of Campylobacter spp. -- Part 2: Colony-count technique and entails the following:

Plating of 1 ml of the packaging swab liquid onto three modified cefoperazone, charcoal deoxycholate agars (CCDA plates: e.g. Oxoid CM739 with Oxoid selective supplement SR155).

Plating of 1 ml of the chicken skin homogenate onto three CCDA plates and 100 µl onto duplicate CCDA plates. Prepare two further 10-fold dilutions in MRD and plate 100 µl of each of these in duplicate onto CCDA plates.

Incubate CCDA plates in a microaerophilic atmosphere at 41.5 ± 1°C for 44 ± 4 h.

Counting and confirmation of suspect/typical colonies

Count plates from those with less than 150 colonies, where possible. As the bacteria rapidly deteriorate in air progress confirmation of colonies immediately. Pick 5 (or less if less present) colonies (based on typical colony morphology) and sub-culture onto Columbia Blood Agar (containing 5 % (v/v) defibrinated blood). Check that growth is

absent after incubation under aerobic conditions after 48 h and check for typical growth in a microaerophilic atmosphere at 41.5 ºC. Confirm oxidase reaction of pure cultures and typical Campylobacter cell morphology (small, slim, curved or spiral, Gram-negative rods/motility (wet mount/phase contrast)). Commercially available latex agglutination test kits can be used to identify campylobacters (e.g. Microscreen® campylobacter (Microgen bioproducts) and Dryspot campylobacter test (Oxoid Ltd) consistent with local Standard Operating Procedures.

Isolates of *Campylobacter* spp. will be sent, as soon as possible, to the Gastrointestinal Bacteria Reference Unit (GBRU) CampyLab, PHE London (Hays DX number DX653008) for speciation and antibiotic resistance testing. One isolate from each positive sample will be sent and archived by GBRU. Isolates sent to GBRU must be clearly labelled with their sample number and the name of the referring laboratory.

A UK WIDE MICROBIOLOGICAL SURVEY OF CAMPYLOBACTER CONTAMINATION IN FRESH WHOLE CHILLED CHICKENS AT RETAIL SALE (Year 2/ 3/ 4)

PROTOCOL

Revised July 2015

Updated 07.07.2015

CONTENTS

ABBREVIATIONS

CCDA-	Charcoal Cefoperazone Deoxycholate Agar
DH -	Department of Health
FSA -	Food Standards Agency
h -	Hour(s)
PHE -	Public Health England
ISO-	International Standards Organisation
LGP-	Laboratory of Gastrointestinal Pathogens
mL -	Millilitres
mm -	Millimetres
s -	Seconds
MS-	Microbiological Services
UKAS-	United Kingdom Accreditation Service

OUTLINE

Background

1. The Food Standards Agency has a key role in preventing foodborne illnesses. The Strategic Plan aims to reduce foodborne disease further and has set a target to reduce Campylobacter contamination in raw chicken.

   Campylobacter is the most prominent bacterium associated with foodborne disease within the United Kingdom. Foodborne Campylobacter is estimated to make more than 280,000 people ill each year in the UK and is the biggest cause of food poisoning. An EFSA Opinion[1] stated that up to 80% of cases can be attributed to raw poultry meat. It is hoped that by reducing the number of highly positive birds through effective control programmes, the number of human cases will decrease.

2. In 2009 the FSA published results of its UK survey of Campylobacter and Salmonella contamination of fresh chicken at retail sale[2], this survey found the prevalence of Campylobacter in chicken at retail in the UK was 65.2% based on the combining of results from the direct plating and enrichment methods. This survey also highlighted the difficulties in isolating the organism and that the presence/absence method had limitations. Literature suggests that using a combination of presence/absence and enumeration testing provides a more robust measure of Campylobacter prevalence. The overall prevalence figure for the survey was therefore determined by combining the Campylobacter positive results from the 927 samples tested by both presence/absence and enumeration methods.

3. In December 2010 The Food Standards Agency, the UK poultry industry and major retailers agreed a new target that will measure efforts to reduce the levels of Campylobacter in chickens at the end of slaughter. There are three categories of contamination levels and, currently, 19% of birds are in the highest category (>1000 cfu/g)[3]. The target is for the industry to reduce the numbers of these most

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[1] **Scientific Opinion on *Campylobacter* in broiler meat production: control options and performance objectives and/or targets at different stages of the food chain: http://www.efsa.europa.eu/en/efsajournal/doc/2105.pdf**

[2] **Food Standards Agency.2009. UK-wide survey of Campylobacter and Salmonella contamination of fresh chicken at retail sale, Food Standards Agency, London.**
http://www.foodbase.org.uk/admintools/reportdocuments/351-1-676_B18025.pdf

[3] **http://www.food.gov.uk/sites/default/files/full-campy-survey-report.pdf**

contaminated birds in UK poultry houses from 27 to 10 % by the end of December 2015. It is estimated that achievement of this target could mean a reduction in Campylobacter food poisoning of up to 50 %. While this target might not be achieved in the timeframe, a significant decline in the Campylobacter levels on whole chickens towards the end of 2015 is expected.

4. In 2014, a UK-wide survey was established to review the levels of Campylobacter on fresh whole retail chickens and also on their packaging. The intention of the survey was to represent a full 12 month period (mid-February 2014 – mid February 2015) and tested a total of 4,011 samples of whole, UK-produced, fresh chicken. Over 19% of the chickens tested were found to contain Campylobacter at a level above 1000 cfu/g. Just under 73% were positive for Campylobacter at any level (i.e. were found to contain Campylobacter at a level above the detectable limit of 10 cfu/g). Just under 7% of the samples were positive for Campylobacter on the outer packaging (i.e. contained Campylobacter at a level above the detectable limit of 10 cfu/g). For 5 out of the 4,005 samples (for which valid results were available for the outer packaging), the level on the outer packaging was found to be above 1000 cfu/g. While a reduction of the most contaminated chickens to the target level of 10% may not be achieved by the end of December 2015, the evidence from retailer trials show promising results that interventions work.

5. This new survey will extend the Year 1 survey for up to an additional 36 months, including yearly breakpoints, and will investigate the prevalence and levels of Campylobacter contamination in fresh whole chilled chickens and on the outside surface of the packaging at retail using the enumeration method, and will provide valuable information on Campylobacter levels post slaughter. Although the survey will take into consideration seasonal fluctuations in Campylobacter prevalence in retail chickens, this is secondary to the surveys primary function to analyse prevalence among retailers. To draw any definitive conclusions regarding seasonality, we will require data from a number of separate years. The continuation of the retail survey for up to another 36 month additionally intends to identify trends as a result of specific retailer initiatives, such as improvements in biosecurity on farm or processing interventions, which among others, include SonoSteam (a technology combining steam and ultrasound to achieve rapid decontamination of food products such as chicken).

Objectives

A. 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.

B. To determine the prevalence and levels of *Campylobacter* spp. contamination found on the outside packaging of samples collected under Objective 1.

C. To identify *Campylobacter* spp. present and determine susceptibility of an agreed percentage of isolates to a defined range of antimicrobial agents.

D. 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.

Publication of results

6. The survey results will be published under the Code of Practice for Official Statistics. This entails restricted access to raw data and pre-release publication documents.

   At the end of the survey, the results and all the information that has been collected about all of the samples taken for the whole survey period will be published on the Agency's website.

   In addition, sample data and results will be reported within four weeks following the end of a sampling month, and each quarter the Agency will publish an interim report (please see Annex 1 for proposed timeline).

Timetable

7. The survey will consist of a 12 month sampling period plus a further 2 months for data analysis and report preparation. The proposed timetable is available in Annex 1.

SURVEY DESIGN

8. Based on information available, the UK core sample per 12 month period will be 4000 samples. These samples will be taken evenly over a 12-month period unless agreed otherwise with the FSA to address additional objectives.

9. The survey design has been modified to increase statistical confidence in the determination of differences in Campylobacter prevalence among retailers. Therefore the aim is to sample an equal number of chickens (100) from each of the main retailers each quarter. This new design, informed by the knowledge gained during the Year 1 baseline study, increases statistical confidence in the validity of results obtained during the rest of the survey. It also enables to report earlier, more robustly, and with greater confidence, on any improvements which are observed in samples from individual retailers. The market share data will be applied to re-weight the dataset when producing annual estimates of the average prevalence of *Campylobacter* within the UK market.

   Based on more recent market share data (purchased from Kantar in February 2015), the number of retailers to be named has been increased by adding Aldi and Lidl to the list of retailers named in Year 1.

10. The contractor will be responsible for ensuring that the appropriate number of samples is collected in accordance with the sampling plan agreed with the FSA. The number of chickens to be sampled from each UK country will be proportional to retailer market share figures of the respective country. If any deviations are necessary these will be noted in the final report. The contractor will ensure that sampling is evenly distributed throughout the period of the survey and is responsible for selecting and collecting samples at random within these criteria. If possible and in agreement between the contractor and the Agency, a maximum of 4 different chicken types (e.g. different size, brand or rearing) will be collected from any one store on any one occasion; the number of samples collected should be reduced if the sampler is unable to collect 4 different chicken types. A maximum of 2 samples should be taken from butchers and smaller independent stores/grocers at any one time.

11. The aim of this survey is to obtain a total of 4000 samples of whole UK produced raw fresh chilled chicken within any 12 month period. Sample numbers should be reviewed every month to ensure that chickens are being sampled according to the agreed sampling plan.

12. The contractor will provide smaller independent retail outlets with a letter from the Agency informing them that samples have been taken from their premises in order to carry out a survey (Annex 2). For larger retail chains (i.e. Tesco, Asda, Sainsbury's etc.) this is not necessary, as the relevant contact at head office will be sent a list of the premises from which samples have been obtained by the Agency.

SAMPLING

Sample collection

13. It is essential that cross-contamination be avoided during the collection of chicken samples. Precautions will therefore be taken at all stages to ensure that the equipment used during sampling, transport and storage is not contaminated with the pathogens investigated in the survey.

14. Contractors will aim to collect samples at random from the refrigerator cabinet and not necessarily from the front of the display. The surface temperature of the chicken should be recorded using an appropriately calibrated Infra-red thermometer, as should information on whether it was displayed in a temperature-controlled environment e.g. chillers and the overall condition of shelving e.g. was there any visible meat juices on the shelf.

    Only packaged whole fresh chilled UK produced birds should be purchased. Unwrapped chickens may be bought but it should be noted on the sampling form and if available, with an indication of the Use-by Date. Samples, which are packaged, must not show evidence of damage. Each sample then should be placed in a separate sampling bag to avoid the risk of cross-contamination during transport and until testing can take place. For chickens collected from retail premises, the sample should not be purchased if the label on the chicken is not clear, does not include the approval number of the slaughterhouse, or is damaged. Chickens from butchers without labels may be sampled only if approval code can be obtained and must be noted down.

15. **Only unseasoned, fresh whole UK produced chilled whole chickens** should be sampled.

    The chickens sampled may be labeled such as: Whole fresh chilled pre-packed UK-produced chicken and may include ones named as “Standard”, “Value”, “Ex Large”, “Large”, “Medium”, “Roaster”, “Small”, “Barn-reared”, “Free-range”, “Roast-in-bag” etc.

    Samples NOT included are:

    - **Frozen whole chickens, portions (whether fresh or frozen) including legs, breast, thigh and wing portions.**
    - **Any ready basted, marinated, seasoned, herbed, stuffed or pre-prepared whole birds.**
    - **Cooked chickens.**

- **Processed chicken products including goujons, nuggets etc..**

16. Standard produced chickens will be sampled as well as a smaller number of free range and organic chickens (sampling of free range and organic chickens is structured to reflect their market share as outlined in Annex 3). A range of chicken weights will be sampled and weights should be noted down and logged in a separate column of the sample detail spreadsheet. Each sample should, at the point of sampling, have at least 2 days remaining on its Use-by Date.

17. When chilled un-packaged chicken is purchased from butchers/independents the sampling officer may need to enquire about the country of origin; if the bird is/or may not be UK-produced it should not be included in the survey. The sampler should ask the butcher for the approval number which should be present on the bulk packaging. Only samples where approval code can be obtained are to be included in the survey.

18. Each sample should be placed in a plastic bag, which is then sealed. Contractors will ensure that samples are kept at between 1 to 8 °C (±1°C) during transportation and kept dry and out of direct sunlight. A data logger should be placed (not in contact with or close to the cool pack) with the samples to monitor compliance with these requirements. If cool packs are used, samples shall not come into direct contact with their surfaces. Samples should not be frozen. Internal air temperature of the temperature controlled unit and package integrity shall be recorded on receipt at the laboratory.

19. It is essential to identify the approval number (used to be known as the health mark) from each sample so that the origin of the chicken can be determined retrospectively.

Sample information

20. All relevant information available from the sample should be recorded on the sample submission form (Annex 4). As far as possible this information should include the name (please ensure consistency throughout all laboratories) and postcode of the retailer, date and time of purchase, the approval number, weight (in a separate column), use-by date, price, product name, packaging information and display temperature. The sampling sheet is completed with the addition of the results from the microbiological testing. This data are then entered onto a spreadsheet compatible with Microsoft Excel (please see Annex 5 for model spreadsheet with required information).

21. Sampling and results should be reviewed every month to ensure that the chickens sampled could generate statistically valid/meaningful results. The samplers should co-ordinate their sampling with the testing laboratory, project manager and the Agency.

TESTING

Receipt of samples

22. On receipt of the samples, laboratories will check the information recorded by the sampler and complete the relevant sections of the laboratory sample submission form. The information will be entered into the Laboratory Information Management System and transferred from there into a spreadsheet compatible with Microsoft Excel or entered directly onto an Excel sheet. Following examination, the product label itself will be removed and stored if intact and readable.

23. Product information will be captured with digital photographs of each chicken in its packaging and the file will be stored and labelled with the appropriate sample number. Photos or scans are to be stored on suitable digital media under the appropriate sample number separated by sampling months. This will be shared with the Agency via Dropbox or other resources. The scan/photograph will be of a high resolution so that all the relevant labelling details are clear. Following examination, the product label itself shall be removed, cleaned and stored if intact and readable.

24. Chickens sampled should reach the laboratory within 24 hours of sampling. In exceptional situations (e.g. long journeys from the Northern Scottish Isles) this period may be extended to within 48 hours; if the transport period was 48 hours from sampling, the sampler must instruct the laboratory to test on receipt. All samples should always be tested before/on their use-by dates.

Examination

25. Samples of chicken will be examined to ensure that the packaging is intact before testing. If packaging has been damaged during transportation this should be noted on the sampling form before testing. The temperature of the samples will also be recorded on receipt. Satisfactory samples will consider the integrity of packaging as well as sample temperature on receipt and only samples deemed satisfactory on receipt will be considered eligible for testing. Satisfactory sample

receipt may be assigned if samples are within the temperature range of 1 to 8°C (±1°C). If the temperature data logger records temperatures below 1 °C at receipt, the temperature of the sample itself would be measured and if this temperature was below 1 °C the sample would only be assigned as satisfactory if the sample temperature was below 1 °C (±1°C) when collected. Similarly, if the temperature data logger records temperatures above 8 °C, the sample itself would be measured and only deemed acceptable if the temperature at receipt is equal to or lower (±1°C) than the temperature when sampled. Sample receipt procedures would also take into account temperature probe uncertainty and transport time. All samples will be delivered before their use-by date.

26. It is essential that handlers take care to avoid cross contamination between samples and between the chicken and its packaging as well as from the surrounding environment at all stages. Gloves must be worn and changed between each sample of chicken. The work-surface of the bench must be sanitised before unwrapping each chicken. Thorough cleaning of equipment and work surfaces will be undertaken regularly. There must be environmental sampling of the laboratory for test bacteria (Campylobacter) during the testing period at regular intervals. The contractor will carry out examinations in areas dedicated to the examination of survey samples and clearly separated from other potentially contaminated materials.

Methodology

27. The microbiological methodology for the testing of each sample (chicken and packaging) for Campylobacter is as follows:

    The quantitative analysis of Campylobacter in a chicken sample will be based on the method described in **EN/ISO/TS 10272-2:2006** 'Microbiology of food and animal feeding stuffs – Horizontal method for detection and enumeration of *Campylobacter* spp Part 2: Colony-count technique' (please see Annex 6 for detailed method).

Data handling and reporting

28. Within four weeks after each sampling month concludes, the contractor will submit to the Agency a progress report that provides details of the samples taken and the Campylobacter counts. The data for antimicrobial resistance profiles for strains isolated will be submitted independently in agreement with the Agency.

29. The contractor is responsible for collating all results and submitting a final report to the Agency. The report will present summary statistics on the prevalence of Campylobacter, together with a breakdown of the species where appropriate. The results should be subjected to detailed statistical analysis by the contractor; these analyses will be agreed with the Agency's Statistics team prior to commencement.

30. All forms, documentation and electronic files must be retained by the contractor until further notice from the Agency in case of issues arising after the completion of the survey. These should be retained for at least 12 month after completion of the current survey. It is not necessary to provide the Agency with hard copies of forms. However, this information must be readily available to the Agency if required.

31. Every month, the sampling numbers will be assessed to ensure that representative samples are being tested to obtain statistically valid/meaningful results. The contractor is responsible for adjusting the sampling plan every month according to any deviations occurring in the previous month(s) while the FSA is responsible for instructing the contractor on any major changes to the sampling strategy e.g. changes in market-share predictions.

Quality Assurance

32. In order to ensure a high level of accuracy in data entry, data checking and backup, the contractor has to be accredited to the relevant ISO methods by an appropriate organisation (e.g. UKAS). The EN/ISO/TS 10272-2 method is currently being revised to become a full standard and any proposed changes in the final draft may be incorporated providing they are within the scope of the accreditation. The contractor must also be able demonstrate satisfactory performance in the testing of food for *Campylobacter* spp. through participation in an independent proficiency testing scheme. The measurement of uncertainty for enumeration of *Campylobacter* spp. must also be determined and the FSA will visit the contractors during the course of the survey to assess how the work is being carried out.

ANNEX 1: PROPOSED TIMETABLE FOR FIRST YEAR OF SURVEY

June 2015	Protocol finalised
June 2015	MoU signed
Quarter 1 (sampling July/ August/ September)	
Beginning July 2015***** *****covering the quarterly sampling months July/ August/ September	Sampling starts
31 August 2015	July sampling data and results to be submitted to Agency
30 September 2015	August sampling data and results to be submitted to Agency
15 October 2015	Agency to receive spreadsheet containing all **sampling** details for Quarter 1
4 November 2015	September results to be updated and submitted to Agency
26 November 2015	Anticipated Quarter 1 publication
Quarter 2 (sampling October/ November/ December)	
30 November 2015	October sampling data and results to be submitted to Agency
4 January 2016	November sampling data and results to be submitted to Agency
18 January 2016	Agency to receive spreadsheet containing all **sampling** details for Quarter 2
3 February 2016	December results to be updated submitted to Agency
25 February 2016	Anticipated Quarter 2 publication
Quarter 3 (sampling January/ February/ March)	
29 February 2016	January sampling data and results to be submitted to Agency
28 March 2016	February sampling data and results to be submitted to Agency
18 April 2016	Agency to receive spreadsheet containing all **sampling** details for Quarter 3
5 May 2016	March results to be updated submitted to Agency
26 May 2016	Anticipated Quarter 3 publication

Updated 07.07.2015

Quarter 4 (sampling April/ May/ June)	
30 May 2016	April sampling data and results to be submitted to Agency
30 June 2016	May sampling data and results to be submitted to Agency
16 July 2016	Agency to receive spreadsheet containing all **sampling** details for Quarter 4
3 August 2016	June results to be updated and submitted to Agency
25 August 2016	Anticipated Quarter 4 publication
1 July 2016	End of sampling period / annual breakpoint
5 September 2016	Final draft report received
29 September 2016	Final report signed off

ANNEX 2: LETTER TO RETAILERS

Letter to be sent to Retailers during Sampling

Insert Council **Logo &/or Name**

<Date>

Dear

This letter has been given to you by an Environmental Health Officer (EHO)/ Sampling Officer (SO) from [insert name of Contractor].

The EHO / SO is authorised by the [Contractor] to carry out food sampling work, and has purchased chicken from your premises as a food sample, which is to be used for a food surveillance survey.

The aim of this particular survey is to ascertain the incidence and contamination level of Campylobacter in raw UK produced chicken available to consumers at retail in the UK. Whole chickens are being sampled and tested during a 12 month period.

This survey is being funded by the Food Standards Agency which has commissioned [name of Contractor] to carry out the sampling.

Your premise has been visited as one of the retail outlets where people may buy raw chicken - the subject of this survey. The raw chicken purchased from your premises will be taken to [insert name of lab] for testing, and you will be provided with the results of this testing by a letter from the Food Standards Agency. Please note that the survey is not for enforcement purposes.

The results of the samples taken in this survey will be collated and will form part of a report on the incidence and contamination level of the pathogen *Campylobacter* spp. on the surface of the packaging and in fresh whole chilled UK chicken on retail sale within the UK. This report will be published by the Food Standards Agency. At the end of the survey, in line with Food Standards Agency policy on openness and transparency in relation to food safety and matters of interest to consumers relative to food, individual retailers/producers of the chicken sampled will be published on the Agency's website www.food.gov.uk as part of this report.

Should you have any queries, please contact Dr Bettina Mavrommatis, Foodborne Disease Control, Food Safety Policy on the following telephone number:
020 7276 8045 or send an E-mail to
Bettina.Mavrommatis@foodstandards.gsi.gov.uk

Yours sincerely

Updated 07.07.2015

ANNEX 3: SAMPLING PLAN

A UK core sample size of approximately 4000 samples of fresh whole UK produced chilled chicken are needed to achieve the precision required. The sample numbers should be reviewed periodically to ensure that statistically meaningful analyses can be carried out.
The sampling will aim to take place evenly over a 12-month period. The sampling plan is structured to reflect market share data sourced from Kantar (February 2015). Sampling will be kept under review and can, as agreed with the FSA, be revised to accommodate any further survey objectives e.g. over-sample during certain periods.

Table 1 Numbers of chickens to be sampled throughout the UK over 12 month

	Total number of chickens
Total UK	4000
England	3250
Scotland	288
Wales	396
Northern Ireland	66

ENGLAND

Retailer groups	Total no. of chickens	No. of Free-range chickens (of total)	No. of Organic chickens (of total)
Tesco	314	30	1
Asda	321	40	1
Sainsbury's	340	28	5
The Co-operative	313	25	0
Morrisons	333	33	1
Waitrose	369	56	12
M&S	310	2	1
Aldi	313	43	0
Lidl	284	13	0
Butchers	186	*	*
Others1	167	66	0

[1]E.g Iceland, Budgens, Costcutter, Nisa, independents, farm shops, markets stalls.
* samples from butchers can be collected from either category (Standard/ Free Range/ Organic) depending on availability

Updated 07.07.2015

WALES

Retailer groups	Total no. of chickens	No. of Free-range chickens (of total)	No. of Organic chickens (of total)
Tesco	49	4	0
Asda	41	3	0
Sainsbury's	32	3	1
The Co-operative	38	4	0
Morrisons	33	3	0
Waitrose	25	6	0
M&S	34	3	1
Aldi	50	6	0
Lidl	66	2	0
Butchers	10	*	*
Others[1]	18	3	0

[1]E.g Iceland, Budgens, Costcutter, Nisa, independents, farm shops, markets stalls.

* samples from butchers can be collected from either category (Standard/ Free Range/ Organic) depending on availability

SCOTLAND

Retailer groups	Total no. of chickens	No. of Free-range chickens (of total)	No. of Organic chickens (of total)
Tesco	26	5	0
Asda	25	3	0
Sainsbury's	13	1	0
The Co-operative	49	5	0
Morrisons	34	4	0
Waitrose	6	1	0
M&S	56	4	0
Aldi	37	3	0
Lidl	29	2	0
Butchers	4	*	*
Others[1]	9	2	

[1]E.g Iceland, Budgens, Costcutter, Nisa, independents, farm shops, markets stalls.

* samples from butchers can be collected from either category (Standard/ Free Range/ Organic) depending on availability

Updated 07.07.2015

NORTHERN IRELAND

Retailer groups	Total no. of chickens	No. of Free-range chickens (of total)	No. of Organic chickens (of total)
Tesco	11	1	0
Asda	13	2	0
Sainsbury's	15	1	0
The Co-operative	0	0	0
Morrisons	0	0	0
Waitrose	0	0	0
M&S	0	1	0
Aldi	0	0	0
Lidl	21	1	0
Butchers and Others*	6	2	0

*E.g Dunnes, Iceland, Budgens, Costcutter, Nisa, independents, farm shops, markets stalls.

ANNEX 4: SAMPLING FORM

Public Health England

FOOD WATER AND ENVIRONMENTAL MICROBIOLOGY LABORATORY, xxxxxx
xxxxxxxxxxxxxxxxxxxxxxxx
xxxxxxxxxxxxxxx
a UKAS accredited Testing Laboratory No.xxxx
Tel: 0xxxxxxxxx
E-mail: LabFwexxxxx@phe.gov.uk

FSA Campylobacter in chickens 2015-2016

RETAILER: (circle which)	ALDI / ASDA / CO-OP / LIDL / M&S MORRISONS / SAINSBURYS TESCO / WAITROSE / BUTCHERS* OTHER*
Post Code:	.....
*If BUTCHERS or OTHER give premises name and address:	....

AFFIX LABORATORY NUMBER HERE

Sampling done by (tick): PHE lab ☐ MH Scientific ☐
CRCE ☐ N Ireland lab ☐
Sender contact tel # .....
Cool Box ID: .....

Sample collected by: ..... Date collected: ..... Time collected (24 hour clock): ..... Temperature at collection .....°C

Sample Details
Sample description (enter full name of product as it appears on the label)
.....
Use by date: dd/mm/yy...../...../..... Batch Number (Approval Number .....)

Additional sample details: Enter answers to the questions and **TICK ALL BOXES THAT APPLY**

Q#	Question	Response
Q1	Display unit	Dry ☐ Wet ☐ Dirty and / or bloody ☐
Q2	Weight of product as shown on packaging	.....Grams
Q3	Price	£.....
Q4	Type of chicken (as shown on packaging)	Standard ☐ Free range ☐ Organic ☐ Choose Standard if type not stated
Q5	Other chicken details	Halal ☐ Corn fed ☐ Other ☐ (give details): .....
Q6	Assurance scheme	Red Tractor ☐ Other ☐ (give details e.g. Freedom Foods): .....
Q7	Packaging details	Wrapped tight ☐ Tray present ☐ Modified atmosphere ☐ Other (give details): .....
Q8	Visible liquid in pack?	Yes ☐ No ☐
Q9	Other sampling comments:	
Q10	**For Lab Use Only**	Weight of neck skin in sample .....Grams

LABORATORY USE ONLY (Record details of unsatisfactory findings in comments)

Date received: ...../...../.....
Time received: .....
Received by: .....
Received from: .....

Data logger / probe ID: .....
Air / In between pack (delete as appropriate)
Temp. on receipt: .....°C

Samples & Receipt: ☐ SATIS ☐ UNSATIS

Comments:

Associate with STARLIMS Study No.:

FSA Campy in chickens 2015 submittal form v1

ANNEX 5: Model data submission template (as Excel)

Country	NI lab ref	Post code	Sample Number	Temp at Collection (Store) °C	Temp on Receipt (Lab) °C	Date Examined	Retailer store	Retailer details (if Others/ Butchers)	Premises Address (Others/ butcher)	Sample Details	chicken weight (g)	Other Packaging Details	Other packaging comments	Chicken type	Specify other production types	Cost of chicken (£)	Approval Number	Use by date	breastskin taken for analysis (g)	Test Name	Result	Units	Speciation	Result
as before	*as before*	*as before*	*as before*	*please specify accurate (without characters)*	*please specify accurate (without characters)*	*consistent date format*	*consistency throughout*			*please specify here*	*number in gram without characters*	*please specify here*	*please specify here*	*specify consistently*	*specify if described by following*	*specify (number only; no characters)*	*specify (number only; no characters)*	*consistent date format*	*please specify breastskin taken for analysis (g)*					
				5	6.2	12/08/2015	Co-op			Small whole chicken Fresh Class A	4269	Roast-in-bag	liquid visible in pack	STD	Halal	2.99	8005	12/08/2015						
							Asda					No tray, modified atmosphere packed		FR	cornfed									
							Morrisons					Tray present, modified atmosphere packed.		O										
							Sainsbury's					No tray. Plastic, lightly wrapped.												
							Tesco					Loose bag. No tray.												
							Lidl																	
							Waitrose																	
							Aldi																	
							M&S																	
							Others	NISA																
								Iceland																
								etc.																
							Butchers	Pat The Butcher	1 Little Aston Lane, Little Aston, Birmingham															

ANNEX 6: LABORATORY METHODOLOGY

Overview

Chicken neck-skin samples and the outside surface of packaging will be analysed for Campylobacter. Wear suitable single-use gloves for handling the packaged chicken, changing gloves after each sample.

Outer packaging swab

Place the wrapped chicken, with the outer bag folded away from the pack label onto a clean surface and take a picture (with sample number and pack label clearly visible) and retain label after examination.
Add 10 ml of Maximum Recovery Diluent (MRD) to a SpongeSicle™swab and ensure the swab is thoroughly wetted.
Remove the outer sample bag and place the wrapped chicken on a previously disinfected dry plastic tray wearing disposable gloves.
Swab the entire outer surface of the chicken packaging using aseptic technique (swab whole pack twice using both side of the swab). In case of 'Roast/ Cook in bag' chicken, which for some retailers can come double-bagged, swab the outer bag for enumeration.
Replace the swab in its bag breaking off the stick and then stomaching the swab for 30 s.
Using a sterile pastette remove > 2 ml into a sterile container for enumeration as described below.

Chicken skin

Wearing a fresh pair of disposable gloves, remove the chicken from its wrapping, taking care not to allow contact between the chicken and outer packaging. Using sterile instruments (e.g. scissors and tweezers) aseptically remove skin from the neck area (if < 25 g neck-skin is available top up with breast skin (**record weight of this**)) to make a 25 g test portion, avoiding fat and place this into a sterile bag.
Add 225 ml BPW and homogenise for one minute. Remove > 3 ml for enumeration as described below.

Enumeration of Campylobacter spp.

Enumerate *Campylobacter* spp. by the surface plate method as described in the PHE Methods - Detection and enumeration of *Campylobacter* spp.: *FNES15 (*F21) v2 (see Appendix I). This method is based ISO/TS 10272-2:2006 Microbiology of food and animal feeding stuffs -- Horizontal method for detection and enumeration of Campylobacter spp. -- Part 2: Colony-count technique and entails the following:

Plating of 1 ml of the packaging swab liquid onto three modified cefoperazone, charcoal deoxycholate agars (CCDA plates: e.g. Oxoid CM739 with Oxoid selective supplement SR155).

Plating of 1 ml of the chicken skin homogenate onto three CCDA plates and 100 µl onto duplicate CCDA plates. Prepare two further 10-fold dilutions in MRD and plate 100 µl of each of these in duplicate onto CCDA plates.

Incubate CCDA plates in a microaerophilic atmosphere at 41.5 ± 1°C for 44 ± 4 h.

Counting and confirmation of suspect/typical colonies

Count plates from those with less than 150 colonies, where possible. As the bacteria rapidly deteriorate in air progress confirmation of colonies immediately. Pick 5 (or less if less present) colonies (based on typical colony morphology) and sub-culture onto Columbia Blood Agar (containing 5 % (v/v) defibrinated blood). Check that growth is

absent after incubation under aerobic conditions after 48 h and check for typical growth in a microaerophilic atmosphere at 41.5 ºC. Confirm oxidase reaction of pure cultures and typical Campylobacter cell morphology (small, slim, curved or spiral, Gram-negative rods/motility (wet mount/phase contrast)). Commercially available latex agglutination test kits can be used to identify campylobacters (e.g. Microscreen® campylobacter (Microgen bioproducts) and Dryspot campylobacter test (Oxoid Ltd) consistent with local Standard Operating Procedures.

Isolates of *Campylobacter* spp. will be sent, as soon as possible, to the Gastrointestinal Bacteria Reference Unit (GBRU) CampyLab, PHE London (Hays DX number DX653008) for speciation and antibiotic resistance testing. One isolate from each positive sample will be sent and archived by GBRU. Isolates sent to GBRU must be clearly labelled with their sample number and the name of the referring laboratory.

A UK WIDE MICROBIOLOGICAL SURVEY OF CAMPYLOBACTER CONTAMINATION IN FRESH WHOLE CHILLED CHICKENS AT RETAIL SALE (Year 3/ 4)

PROTOCOL

Revised August 2016

Updated 01.08.2016

CONTENTS

ABBREVIATIONS

BPW-	Buffered Peptone Water
CCDA-	Charcoal Cefoperazone Deoxycholate Agar
DH -	Department of Health
FSA -	Food Standards Agency
h -	Hour(s)
PHE -	Public Health England
ISO-	International Standards Organisation
GBRU-	Gastrointestinal Bacteria Reference Unit
mL -	Millilitres
mm -	Millimetres
s -	Seconds
MS-	Microbiological Services
UKAS-	United Kingdom Accreditation Service

OUTLINE

Background

1. The Food Standards Agency has a key role in preventing foodborne illnesses. The Strategic Plan aims to reduce foodborne disease further and has set a target to reduce Campylobacter contamination in whole raw chicken.

   Campylobacter is the most prominent bacterium associated with foodborne disease within the United Kingdom. Foodborne Campylobacter is estimated to make more than 280,000 people ill each year in the UK and is the biggest cause of food poisoning. An EFSA Opinion[1] stated that up to 80 % of cases can be attributed to raw poultry meat. It is hoped that by reducing the number of highly positive birds through effective control programmes, the number of human cases will decrease.

2. In December 2010, the Food Standards Agency, the UK poultry industry and major retailers agreed a new target that will measure efforts to reduce the levels of Campylobacter in chickens at the end of slaughter. The target is for the industry to reduce the numbers of most contaminated birds (>1000 cfu/g) in UK poultry houses from 27 to 10 % by the end of 2015 (this deadline has now been extended to 2016). It is estimated that achievement of this target could mean a reduction in Campylobacter food poisoning of up to 50 %.

3. In 2014, a UK-wide survey was established to review the levels of Campylobacter on fresh whole retail chickens and also on their packaging. The intention of the survey was to represent a full 12 month period (mid-February 2014 – mid February 2015) and tested a total of 4,011 samples of whole, UK-produced, fresh chicken. Over 19 % of the chickens tested were found to contain Campylobacter at a level above 1000 cfu/g. Just under 73 % were positive for Campylobacter at any level (i.e. were found to contain Campylobacter at a level above the detectable limit of 10 cfu/g). Just under 7 % of the samples were positive for Campylobacter on the outer packaging (i.e. contained Campylobacter at a level above the detectable limit of 10 cfu/g). For 5 out of the 4,005 samples (for which valid results were available for the outer packaging), the level on the outer packaging was found to be

[1] **Scientific Opinion on *Campylobacter* in broiler meat production: control options and performance objectives and/or targets at different stages of the food chain: http://www.efsa.europa.eu/en/efsajournal/doc/2105.pdf**

above 1000 cfu/g. While a reduction of the most contaminated chickens to the target level of 10 % was not achieved by the end of December 2015, the evidence from retailer trials showed promising results that interventions work and the target was rolled forward to the end of 2016.

4. In July 2015, Survey Year 2 commenced under a revised sampling frame that included sampling of equal numbers of chickens from the 9 major retailers (100 chickens per retailer per sampling quarter). This equal sampling aided the ability to compare fairly between retailers. Survey Year 2 was partly completed due to the need for revising the test protocol in-year, but the data from the first 3 quarters (July 2015 – March 2016) suggested contamination rates were further decreasing (FSA 2016[2]).

5. Thus far, the laboratory protocol for detecting Campylobacter involved measuring the amount of Campylobacter on 25g of chicken neck skin (generally the most contaminated part of the bird) that could be topped up with breast skin if 25g was not achieved by neck skin only. During Survey Year 2, processors have increasingly trimmed back larger parts of the neck skin. While good news for the consumer, since this reduces the amount of Campylobacter on the bird, it also introduced a greater variation between the samples in our survey as the ratio of neck skin: breast skin in samples was variable. This hampered fair retailer to retailer comparisons and accurate comparisons with previous results.

6. The FSA therefore suspended the retail survey in April 2016 for the duration of 4 months while establishing a new testing methodology at Public Health England with the aim to restore the robustness of the survey. In the final quarter of Survey Year 2 a new protocol was developed to ensure the best possible sample was identified for the continued monitoring of campylobacters in retail chicken. The protocol presented in this document is the result of the evaluation of several methodologies and considered to provide the most robust comparison of campylobacter contamination of retail chicken.

7. Additionally, with regards to packaging contamination, recent evidence suggests a significant decline in Campylobacter levels on the outside of the chicken packaging. In the final quarter of Year 2, Campylobacter was not detected in over 95% of outer packaging (Jan 16 - Mar 16) and only 1 of the 1004 samples was found to have the highest levels of

[2] Campylobacter contamination in fresh whole chilled UK-produced chickens at retail: January – March 2016: http://www.food.gov.uk/sites/default/files/campy-survey-report-jan-mar-2016.pdf

Campylobacter (more than 1000 cfu/swab) contamination. Hence, detection of Campylobacter levels on the chicken packaging was suspended and does not form part of the new protocol.

8. This new survey will extend the previous surveys for up to an additional 24 months, including a breakpoint after 12 months, and will investigate the prevalence and levels of Campylobacter contamination in fresh whole chilled chickens at retail using the enumeration method.
   Although the survey will take into consideration seasonal fluctuations in Campylobacter prevalence in retail chickens, this is secondary to the survey's primary function to analyse prevalence among retailers. To draw any definitive conclusions regarding seasonality, we will require data from a number of separate years. The continuation of the retail survey for up to another 24 months additionally intends to identify trends as a result of specific retailer initiatives, such as improvements in biosecurity on farm or processing interventions.

Objectives

1. 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 24 month period, including annual breakpoints.

2. To identify *Campylobacter* spp. present and determine susceptibility of an agreed percentage of isolates to a defined range of antimicrobial agents.

3. 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.

Publication of results

9. The survey results will be published under the Code of Practice for Official Statistics. This entails restricted access to raw data and pre-release publication documents.
   At the end of the survey, the results and all the information that has been collected about all of the samples taken for the whole survey period will be published on the Agency's website.
   In addition, sample data and results will be reported within four weeks following the end of a sampling month to the Agency. At predefined time points the Agency will publish interim reports.

Timetable

10. The survey will consist of a 12 month sampling period plus a further 2 months for data analysis and report preparation.

SURVEY DESIGN

11. Based on information available, the UK core sample per 12 month period will be 4000 samples. These samples will be taken evenly over a 12-month period unless agreed otherwise with the FSA to address additional objectives.

12. An equal number of chickens (100) will be sampled from each of the nine main retailers each quarter. This design, informed by the knowledge gained during the Year 1 and 2 baseline study, increases statistical confidence in the validity of results obtained during the survey. It also enables to report earlier, more robustly, and with greater confidence, on any improvements which are observed in samples from individual retailers. The market share data, obtained from Kantar in March 2015, will be applied to re-weight the dataset when producing estimates of the average prevalence of *Campylobacter* within the UK market.

13. The contractor will be responsible for ensuring that the appropriate number of samples is collected in accordance with the sampling plan agreed with the FSA. The number of chickens to be sampled from each UK country will be proportional to retailer market share figures of the respective country. If any deviations are necessary these will be noted in the final report. The contractor will ensure that sampling is evenly distributed throughout the period of the survey and is responsible for selecting and collecting samples at random within these criteria. If possible and in agreement between the contractor and the Agency, a maximum of 4 different chicken types (e.g. different size, brand or rearing) will be collected from any one store on any one occasion; the number of samples collected should be reduced if the sampler is unable to collect 4 different chicken types. A maximum of 2 samples should be taken from butchers and smaller independent stores/grocers at any one time.

14. The aim of this survey is to obtain a total of 4000 samples of whole UK produced raw fresh chilled chicken within any 12 month period. Sample numbers should be reviewed every month to ensure that chickens are being sampled according to the agreed sampling plan.

15. The contractor will provide smaller independent retail outlets with a letter from the Agency informing them that samples have been taken from their premises in order to carry out a survey (Annex 1). For larger retail chains (i.e. Tesco, Asda, Sainsbury's etc.) this is not necessary,

as the relevant contact at head office will be sent a list of the premises from which samples have been obtained by the Agency.

SAMPLING

Sample collection

16. It is essential that cross-contamination be avoided during the collection of chicken samples. Precautions will therefore be taken at all stages to ensure that the equipment used during sampling, transport and storage is not contaminated with the pathogens investigated in the survey.

17. Contractors will aim to collect samples at random from the refrigerator cabinet and not necessarily from the front of the display. The surface temperature of the chicken should be recorded using an appropriately calibrated Infra-red thermometer, as should information on whether it was displayed in a temperature-controlled environment e.g. chillers and the overall condition of shelving e.g. was there any visible meat juices on the shelf.

    Only packaged whole fresh chilled UK produced birds should be purchased. Unwrapped chickens may be bought but it should be noted on the sampling form and if available, with an indication of the Use-by Date. Samples, which are packaged, must not show evidence of damage. Each sample then should be placed in a separate sampling bag to avoid the risk of cross-contamination during transport and until testing can take place. For chickens collected from retail premises, the sample should not be purchased if the label on the chicken is not clear, does not include the approval number of the slaughterhouse, or is damaged. Chickens from butchers without labels may be sampled only if approval code can be obtained and must be noted down.

18. **Only unseasoned, fresh whole UK produced chilled whole chickens** should be sampled.

    The chickens sampled may be labeled such as: Whole fresh chilled pre-packed UK-produced chicken and may include ones named as “Standard”, “Value”, “Ex Large”, “Large”, “Medium”, “Roaster”, “Small”, “Barn-reared”, “Free-range”, “Roast-in-bag” etc.

    Samples NOT included are:

    - **Frozen whole chickens, portions (whether fresh or frozen) including legs, breast, thigh and wing portions.**

- **Any ready basted, marinated, seasoned, herbed, stuffed or pre-prepared whole birds.**
- **Cooked chickens.**
- **Processed chicken products including goujons, nuggets etc..**

19. Standard produced chickens will be sampled as well as a smaller number of free range and organic chickens (sampling of free range and organic chickens is structured to reflect their market share as outlined in Annex 2). A range of chicken weights will be sampled and weights should be noted down and logged in a separate column of the sample detail spreadsheet. Each sample should, at the point of sampling, have at least 2 days remaining on its Use-by Date.

20. When chilled un-packaged chicken is purchased from butchers/independents the sampling officer may need to enquire about the country of origin; if the bird is/or may not be UK-produced it should not be included in the survey. The sampler should ask the butcher for the approval number which should be present on the bulk packaging. Only samples where approval code can be obtained are to be included in the survey.

21. Each sample should be placed in a plastic bag, which is then sealed. Contractors will ensure that samples are kept at between 1 to 8 °C (±1°C) during transportation and kept dry and out of direct sunlight. A data logger should be placed (not in contact with or close to the cool pack) with the samples to monitor compliance with these requirements. If cool packs are used, samples shall not come into direct contact with their surfaces. Samples should not be frozen. Internal air temperature of the temperature controlled unit and package integrity shall be recorded on receipt at the laboratory.

22. It is essential to identify the approval number (used to be known as the health mark) from each sample so that the origin of the chicken can be determined retrospectively.

Sample information

23. All relevant information available from the sample should be recorded on the sample submission form (Annex 3). As far as possible this information should include the name (please ensure consistency throughout all laboratories) and postcode of the retailer, date and time of purchase, the approval number, weight (in a separate column), use-by date, price, product name, packaging information and display

temperature. The sampling sheet is completed with the addition of the results from the microbiological testing. This data are then entered onto a spreadsheet compatible with Microsoft Excel (please see Annex 4 for model spreadsheet with required information).

24. Sampling and results should be reviewed every month to ensure that the chickens sampled could generate statistically valid/meaningful results. The samplers should co-ordinate their sampling with the testing laboratory, project manager and the Agency.

TESTING

Receipt of samples

25. On receipt of the samples, laboratories will check the information recorded by the sampler and complete the relevant sections of the laboratory sample submission form. The information will be entered into the Laboratory Information Management System and transferred from there into a spreadsheet compatible with Microsoft Excel or entered directly onto an Excel sheet. Following examination, the product label itself will be removed and stored if intact and readable.

26. Product information will be captured with digital photographs of each chicken in its packaging and the file will be stored and labelled with the appropriate sample number. Photos or scans are to be stored on suitable digital media under the appropriate sample number separated by sampling months. This will be shared with the Agency via Dropbox or other resources. The scan/photograph will be of a high resolution so that all the relevant labelling details are clear. Following examination, the product label itself shall be removed, cleaned and stored if intact and readable.

27. Chickens sampled should reach the laboratory within 24 hours of sampling. In exceptional situations (e.g. long journeys from the Northern Scottish Isles) this period may be extended to within 48 hours; if the transport period was 48 hours from sampling, the sampler must instruct the laboratory to test on receipt. All samples should always be tested before/on their use-by dates.

Examination

28. Samples of chicken will be examined to ensure that the packaging is intact before testing. If packaging has been damaged during

transportation this should be noted on the sampling form before testing. The temperature of the samples will also be recorded on receipt. Satisfactory samples will consider the integrity of packaging as well as sample temperature on receipt and only samples deemed satisfactory on receipt will be considered eligible for testing. Satisfactory sample receipt may be assigned if samples are within the temperature range of 1 to 8 °C (± 1 °C). If the temperature data logger records temperatures below 1 °C at receipt, the temperature of the sample itself would be measured and if this temperature was below 1 °C the sample would only be assigned as satisfactory if the sample temperature was below 1 °C (± 1°C) when collected. Similarly, if the temperature data logger records temperatures above 8 °C, the sample itself would be measured and only deemed acceptable if the temperature at receipt is equal to or lower (± 1 °C) than the temperature when sampled. Sample receipt procedures would also take into account temperature probe uncertainty and transport time. All samples will be delivered before their use-by date.

29. It is essential that handlers take care to avoid cross contamination between samples and between the chicken and its packaging as well as from the surrounding environment at all stages. Gloves must be worn and changed between each sample of chicken. The work-surface of the bench must be sanitised before unwrapping each chicken. Thorough cleaning of equipment and work surfaces will be undertaken regularly. There must be environmental sampling of the laboratory for the test bacteria (Campylobacter) during the testing period at regular intervals. The contractor will carry out examinations in areas dedicated to the examination of survey samples and clearly separated from other potentially contaminated materials.

Methodology

30. The microbiological methodology for the testing of each sample for Campylobacter is as follows:
    The quantitative analysis of Campylobacter in a chicken sample will be based on the method described in **EN/ISO/TS 10272-2:2006** 'Microbiology of food and animal feeding stuffs – Horizontal method for detection and enumeration of *Campylobacter* spp Part 2: Colony-count technique' (please see Annex 5 for detailed method).

Data handling and reporting

31. Within four weeks after each sampling month concludes, the contractor will submit to the Agency a progress report that provides details of the

samples taken and the Campylobacter counts. The data for antimicrobial resistance profiles for strains isolated will be submitted independently in agreement with the Agency.

32. The contractor is responsible for collating all results and submitting a final report to the Agency. The report will present summary statistics on the prevalence of Campylobacter, together with a breakdown of the species where appropriate. The results should be subjected to detailed statistical analysis by the contractor; these analyses will be agreed with the Agency's Statistics team prior to commencement.

33. All forms, documentation and electronic files must be retained by the contractor until further notice from the Agency in case of issues arising after the completion of the survey. These should be retained for at least 12 months after completion of the current survey. It is not necessary to provide the Agency with hard copies of forms. However, this information must be readily available to the Agency if required.

34. Every month, the sampling numbers will be assessed to ensure that representative samples are being tested to obtain statistically valid/meaningful results. The contractor is responsible for adjusting the sampling plan every month according to any deviations occurring in the previous month(s) while the FSA is responsible for instructing the contractor on any major changes to the sampling strategy e.g. changes in market-share predictions.

Quality Assurance

35. In order to ensure a high level of accuracy in data entry, data checking and backup, the contractor has to be accredited to the relevant ISO methods by an appropriate organisation (e.g. UKAS). The EN/ISO/TS 10272-2 method is currently being revised to become a full standard and any proposed changes in the final draft may be incorporated providing they are within the scope of the accreditation. The contractor must also be able demonstrate satisfactory performance in the testing of food for *Campylobacter* spp. through participation in an independent proficiency testing scheme. The measurement of uncertainty for enumeration of *Campylobacter* spp. must also be determined and the FSA will visit the contractors during the course of the survey to assess how the work is being carried out.

ANNEX 1: LETTER TO RETAILERS

Letter to be sent to Retailers during Sampling

Insert Council **Logo &/or Name**

<Date>

Dear

This letter has been given to you by an Environmental Health Officer (EHO)/ Sampling Officer (SO) from [insert name of Contractor].

The EHO / SO is authorised by the [Contractor] to carry out food sampling work, and has purchased chicken from your premises as a food sample, which is to be used for a food surveillance survey.

The aim of this particular survey is to ascertain the incidence and contamination level of Campylobacter in raw UK produced chicken available to consumers at retail in the UK. Whole chickens are being sampled and tested during a 12 month period.

This survey is being funded by the Food Standards Agency which has commissioned [name of Contractor] to carry out the sampling.

Your premise has been visited as one of the retail outlets where people may buy raw chicken - the subject of this survey. The raw chicken purchased from your premises will be taken to [insert name of lab] for testing, and you will be provided with the results of this testing by a letter from the Food Standards Agency. Please note that the survey is not for enforcement purposes.

The results of the samples taken in this survey will be collated and will form part of a report on the incidence and contamination level of the pathogen *Campylobacter* spp. on fresh whole chilled UK chicken on retail sale within the UK. This report will be published by the Food Standards Agency. At the end of the survey, in line with Food Standards Agency policy on openness and transparency in relation to food safety and matters of interest to consumers relative to food, individual retailers/producers of the chicken sampled will be published on the Agency's website www.food.gov.uk as part of this report.

Should you have any queries, please contact Dr Bettina Mavrommatis, Foodborne Disease Control, Food Safety Policy on the following telephone number:
020 7276 8045 or send an E-mail to
Bettina.Mavrommatis@foodstandards.gsi.gov.uk

Yours sincerely

Updated 01.08.2016

ANNEX 2: SAMPLING PLAN

A UK core sample size of approximately 4000 samples of fresh whole UK produced chilled chicken are needed to achieve the precision required. The sample numbers should be reviewed periodically to ensure that statistically meaningful analyses can be carried out. The sampling will aim to take place evenly over a 12-month period. The sampling plan is structured to reflect market share data sourced from Kantar. Sampling will be kept under review and can, as agreed with the FSA, be revised to accommodate any further survey objectives e.g. over-sample during certain periods.

Table 2 Samples in relation to retailer and country

	England			Scotland			Wales			NI			All
	Standard	Free Range	Organic	Standard	Free Range	Organic	Standard	Free Range	Organic	Standard	Free Range	Organic	
Tesco	283	30	1	21	5	0	45	4	0	10	1	0	400
Sainsbury's	307	28	5	12	1	0	28	3	1	14	1	0	400
Morrisons	299	33	1	30	4	0	30	3	0	0	0	0	400
Aldi	270	43	0	34	3	0	44	6	0	0	0	0	400
Asda	280	40	1	22	3	0	38	3	0	11	2	0	400
The Co-Operative	288	25	0	44	5	0	34	4	0	0	0	0	400
Lidl	271	13	0	27	2	0	64	2	0	20	1	0	400
Waitrose	301	56	12	5	1	0	19	6	0	0	0	0	400
Marks & Spencer	287	22	1	52	4	0	30	3	1	0	0	0	400
Butchers[1]	186			4			10			0			200
Others[2]	101	66	0	7	2	0	15	3	0	4	2	0	200

[1]Samples from butchers can be collected from either category (Standard/ Free Range/ Organic) depending on availability. [2] e.g Iceland, Budgens, Costcutter, Nisa, independents, farm shops, markets stalls.

Updated 01.08.2016

ANNEX 3: SAMPLING FORM

Public Health England

FOOD WATER AND ENVIRONMENTAL MICROBIOLOGY LABORATORY, xxxxxx
xxxxxxxxxxxxxxxxxxxxxxxxxxxx,
xxxxxxxxxxxxxxxxxx
a UKAS accredited Testing Laboratory No.xxxx
Tel: 0xxxxxxxxx
E-mail: LabFwexxxxx@phe.gov.uk

FSA Campylobacter in chickens 2016-2017

Premises name: (circle which)	ALDI / ASDA / CO-OP / LIDL / M&S MORRISONS / SAINSBURYS TESCO / WAITROSE / BUTCHERS* OTHER*	**AFFIX LABORATORY NUMBER HERE**	
Post Code:	.....	Senders reference (tick):	PHElab ☐ MH Scientific ☐
*If BUTCHERS or OTHER give premises name and address:	....	Sender contact tel #	CRCE ☐ AFBIlab ☐ .....
		Cool Box ID:	.....

Sampling officer ID:	.....	Date collected:	.....	Time collected (24 hour clock):	.....	Temperature at collection	.....°C

Sample Details
Sample description (enter full name of product as it appears on the label)

.....

Use by date: dd/mm/yy ...../...../..... Batch Number (Approval Number): 

Additional sample details: Enter answers to the questions and TICK ONE BOX per question

Q#	Question	Response
Q1	Weight of product as shown on packaging	.....Grams
Q2	Price	£.....
Q3	Type of chicken (as shown on packaging – Tick 'Standard' if not known	Standard ☐ Free range ☐ Organic ☐
Q4	Other chicken details	Halal ☐ Corn fed ☐ Other ☐ (give details): .....
Q5	Assurance scheme	Red Tractor only ☐ Other ☐ (e.g. Freedom Foods): .....
Q6	Packaging atmosphere	Modified atmosphere ☐ NOT modified atmosphere ☐
Q7	Packaging other details	Roast-in-bag ☐ Other ☐(e.g. wrapped tight /tray present): ....
Q8	Visible liquid inside pack?	Yes ☐ No ☐
Q9	Other sampling comments	.....
Q10	For Lab Use Only	Weight of neck skin in sample .....Grams

LABORATORY USE ONLY (Record details of unsatisfactory findings in comments)

Date received: ...../...../.....
Time received: .....
Received by: .....
Received from: .....

Data logger / probe ID: .....
Air / In between pack (delete as appropriate)
Temp. on receipt:
.....°C

Samples & Receipt: ☐ SATIS ☐ UNSATIS

Comments:
.....
On STARLIMS associate with Study:
Campylobacter in chicken study (2016-17)

FSA Campy in chickens 2015 submittal form v1

Updated 01.08.2016

ANNEX 4: MODEL DATA SUBMISSION TEMPLATE (AS EXCEL)

Country	NI lab ref	Post code	Sample Number	Temp at Collection (Store) °C	Temp on Receipt (Lab) °C	Date Examined	Retailer store	Retailer details (if Others/ Butchers)	Premises Address (Others/ butcher)	Sample Details	chicken weight (g)	Other Packaging Details	Other packaging comments	Chicken type	Specify other production types	Cost of chicken (£)	Approval Number	Use by date	Neck skin weight (g)	Result	Units	Speciation	Result
as before	*as before*	*as before*	*as before*	*please specify accurate (without characters)*	*please specify accurate (without characters)*	*consistent date format*	*consistency throughout*			*please specify here*	*number in gram without characters*	*please specify here*	*please specify here*	*specify consistently*	*specify if described by following*	*specify (number only; no characters)*	*specify (number only; no characters)*	*consistent date format*	*please specify neck skin taken for analysis (g) Value between 2g<neck skin>10g*				
				5	6.2	12/08/2015	Co-op			Small whole chicken Fresh Class A	4269	Roast-in-bag	liquid visible in pack	STD	Halal	2.99	8005	12/08/2015					
							Asda					No tray, modified atmosphere packed		FR	cornfed								
							Morrisons					Tray present, modified atmosphere packed.		O									
							Sainsbury's					No tray. Plastic, lightly wrapped.											
							Tesco					Loose bag. No tray.											
							Lidl																
							Waitrose																
							Aldi																
							M&S																
							Others	NISA															
								Iceland															
								etc.															
							Butchers	Pat The Butcher	1 Little Aston Lane, Little Aston, Birmingham														

ANNEX 5: LABORATORY METHODOLOGY

Overview

Chicken samples will be analysed for Campylobacter. Wear suitable single-use gloves for handling the packaged chicken, changing gloves after each sample.

Chicken skin sampling

Wearing a fresh pair of disposable gloves, remove the chicken from its wrapping, taking care not to allow contact between the chicken and outer packaging. Using sterile instruments (e.g. scissors and tweezers) aseptically remove 10 g skin from the neck area (if 10 g of neck skin is not available, a range of 2 to 10 g can be used, but weight needs to be accurately recorded), avoiding fat and place this into a sterile bag. Add BPW so that a ratio of 1 part chicken skin weight to 9 parts BPW weight is achieved and homogenise for one minute. Remove > 3 ml for enumeration as described below. Chickens with < 5 g neck-skin available for testing must be re-sampled and tested. Chickens with neck skin weights of 2g-5g will be analysed according to protocol, but results not published.

Enumeration of Campylobacter spp.

Enumerate *Campylobacter* spp. by the surface plate method as described in the PHE Methods - Detection and enumeration of *Campylobacter* spp.: *FNES15 (*F21) v2. This method is based ISO/TS 10272-2:2006 Microbiology of food and animal feeding stuffs -- Horizontal method for detection and enumeration of Campylobacter spp. -- Part 2: Colony-count technique and entails the following:

Plating of 1 ml of the chicken skin homogenate onto three modified cefoperazone, charcoal deoxycholate agars (CCDA plates: e.g. Oxoid CM739 with Oxoid selective supplement SR155) and 100 µl onto duplicate CCDA plates. Prepare two further 10-fold dilutions in MRD and plate 100 µl of each of these in duplicate onto CCDA plates.

Incubate CCDA plates in a microaerophilic atmosphere at 41.5 ± 1°C for 44 ± 4 h.

Counting and confirmation of suspect/typical colonies

Count plates from those with less than 150 colonies, where possible. As the bacteria rapidly deteriorate in air progress confirmation of colonies immediately. Pick 5 (or less if less present) colonies (based on typical colony morphology) and sub-culture onto Columbia Blood Agar (containing 5 % (v/v) defibrinated blood). Check that growth is absent after incubation under aerobic conditions after 48 h and check for typical growth in a microaerophilic atmosphere at 41.5 ºC. Confirm oxidase reaction of pure cultures and typical Campylobacter cell morphology (small, slim, curved or spiral, Gram-negative rods/motility (wet mount/phase contrast)). Commercially available latex agglutination test kits can be used to identify campylobacters (e.g. Microscreen® campylobacter (Microgen bioproducts) and Dryspot campylobacter test (Oxoid Ltd) consistent with local Standard Operating Procedures.

Isolates of *Campylobacter* spp. will be sent, as soon as possible, to the Gastrointestinal Bacteria Reference Unit (GBRU) CampyLab, PHE London (Hays DX number DX653008) for speciation and antibiotic resistance testing. One isolate from each positive sample will be sent and archived by GBRU. Isolates sent to GBRU must be clearly labelled with their sample number and the name of the referring laboratory.