Public Health England

FSA Project FS241044

Survey report

A Microbiological survey of campylobacter contamination in fresh whole UK produced chilled chickens at retail sale (2014-15)

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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
MRD	Maximum Recovery Diluent
n	Number
OR	Odds Ratio
SOP	Standard Operating Procedures
spp.	Species
UK	United Kingdom
UKAS	United Kingdom Accreditation Service

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EXECUTIVE SUMMARY

Campylobacter spp. is the most common bacterial cause of foodborne illness in the UK, with chicken considered 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, by the end of 2015. This UK-wide survey was undertaken to determine the levels of *Campylobacter* spp. on whole fresh retail chickens and their packaging.

The survey tested 4,011 samples of whole, UK-produced, fresh chicken from February 2014 to March 2015. The samples were evenly distributed throughout the year and 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 in proportion to their market share, according to available data, with the share of free-range and organic chickens taken into account. The first summary of data from the full survey was published online by the FSA on the 28th May 2015 (FSA 2015a) and this report represents further analysis of the full dataset.

For this retail survey, the chickens were examined 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). 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 (prepared by examining a sponge swab that had been rubbed over the entire outer packaging of the chicken).

The prevalence of *Campylobacter* spp. in the fresh chicken at retail in the UK found through this study was 73.3 %. A significant proportion (19.4 %) of samples had > 1000 cfu/g of 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 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/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/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.

A significant proportion of chicken on sale in the UK remains contaminated therefore *Campylobacter* spp. in chicken continues to be important in terms of foodborne disease risk.

1. 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* and a decrease in the exposure levels from this source is likely to reduce the number of human *Campylobacter* cases. 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) has agreed with industry to reduce campylobacter contamination in raw chicken and has issued a target for this reduction as a measure of the effectiveness of the FSA's Campylobacter Risk Management Programme (FSA 2009; 2010). The target aims 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 10 % by December 2015. The baseline was determined in 2008 using data obtained as part of an EU survey of campylobacters on broiler carcasses where overall 87 % of the UK-produced chickens (testing approximately 400 carcasses) were positive for *Campylobacter* spp.. Such a reduction would 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* spp. 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 whether or not the chicken itself was colonised 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 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, Hutchinson *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 (Hutchinson *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 target *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 identified 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 *C. jejuni*, while one third was *Campylobacter 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, the method has been proven to be quick and reliable using species specific genes (Best *et al*. 2003, Melero *et al*. 2011).

Packaging of raw chicken has been identified as a possible source of *Campylobacter* spp.. 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. However, more quantitative data are needed for *Campylobacter* spp. presence on outer packaging to allow a better risk assessment to be made.

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*, under the umbrella of the Acting on *Campylobacter* Together (ACT) campaign (FSA 2015b).

The aims of FSA Project FS241044 were to:

- Establish a sampling plan to provide enumeration data from retail chickens taking into account the need to compare with data obtained from chickens at the end of the slaughter-line.
- Establish a survey protocol that would provide quality assured enumeration data from retail chickens and their outer-packaging.
- Determine levels of *Campylobacter* spp. in skin samples from 4,000 whole raw UK-produced chilled chickens.
- Determine levels of *Campylobacter* spp. on the outer packaging of 4,000 whole UK-produced chilled chickens.
- Undertake statistical analysis of the distribution of counts of *Campylobacter* spp. from the chicken and outer packaging samples determine significant factors affecting the distributions.
- Ascertain the proportion of strains isolated that are *Campylobacter jejuni*, *C. coli* or other *Campylobacter* spp.

2. METHODS

The survey protocol agreed with the FSA was used for sampling and testing procedures (FSA 2014). The complete FSA Campylobacter Retail survey protocol can be accessed at:-
www.food.gov.uk/sites/default/files/Campylobacter%20in%20Chicken%20PROTOCOL%20FINAL%20with%20amends%20Mar14.pdf

2.1 Sampling methods

Retail outlets were sampled based on market share data from Kantar (Kantar World Panel, 2010). Sampling was spread across all of the UK (reflecting population sizes) and a summary of numbers of samples tested is provided in Appendix I. Samples were collected between February 2014 and March 2015 by trained individuals, who purchased samples from retail outlets. Sampling plans dictated the numbers of whole raw chickens of different production types that should be sampled. Samples were transported according to the study protocol.

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 testing for the survey; five PHE Food, Water and Environmental Microbiology Service Laboratories and the Agri-Food & Biosciences Institute, Belfast. All laboratories used methods based on EN/ISO 10272-2 for the enumeration of *Campylobacter* spp. as detailed in the FSA survey protocol (FSA 2014).
All laboratories followed the defined survey protocol using mCCDA as the primary plating medium. Five laboratories sourced pre-poured mCCDA media from Thermo-Fisher Scientific and the sixth site also used mCCDA made from media powders and supplements sourced from Thermo-Fisher Scientific and produced by an independent media department within their organisation under ISO 9001 standards. All participating laboratories used the same method of achieving a microaerophilic atmosphere.

2.3 Quality Assurance

A pilot study of 400 samples was initiated before commencing this project with the aim to establish and validate methods for sampling and enumerating *Campylobacter* spp. in samples from fresh whole retail chickens and their packaging (Appendix II). 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 g 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 has been used to assess whether any associations can be explained as a result of confounding by other important predictors of contamination. Two different outcome variables have been used; one constructed around the FSA reduction target with the “positive” outcome defined as >1000 cfu per g (and referred to as the high level contamination in this report), and a “negative” outcome being 1000 or fewer cfu per g, the other outcome used has a “positive” outcome defined as the presence of any campylobacters i.e. 10 or more 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 season. The factors; retail cost, temperature on collection, temperature at receipt, and the proportion of breast skin in the test sample were considered in addition in the regression model to asses if important.

In addition, an exploration of effect modification was performed by inclusion of interactions between the predictor variables. This provides an investigation of whether the proportion with *Campylobacter* contamination for each category of a predictor variable, depends on other factors. These results have not been present in the regression results but are described. No post-hoc weighting for retailers market share was applied to any of the statistical analyses presented in this report.

3. RESULTS

Fresh raw whole UK produced chickens were collected from retail outlets across the UK between February 2014 and March 2015 (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.

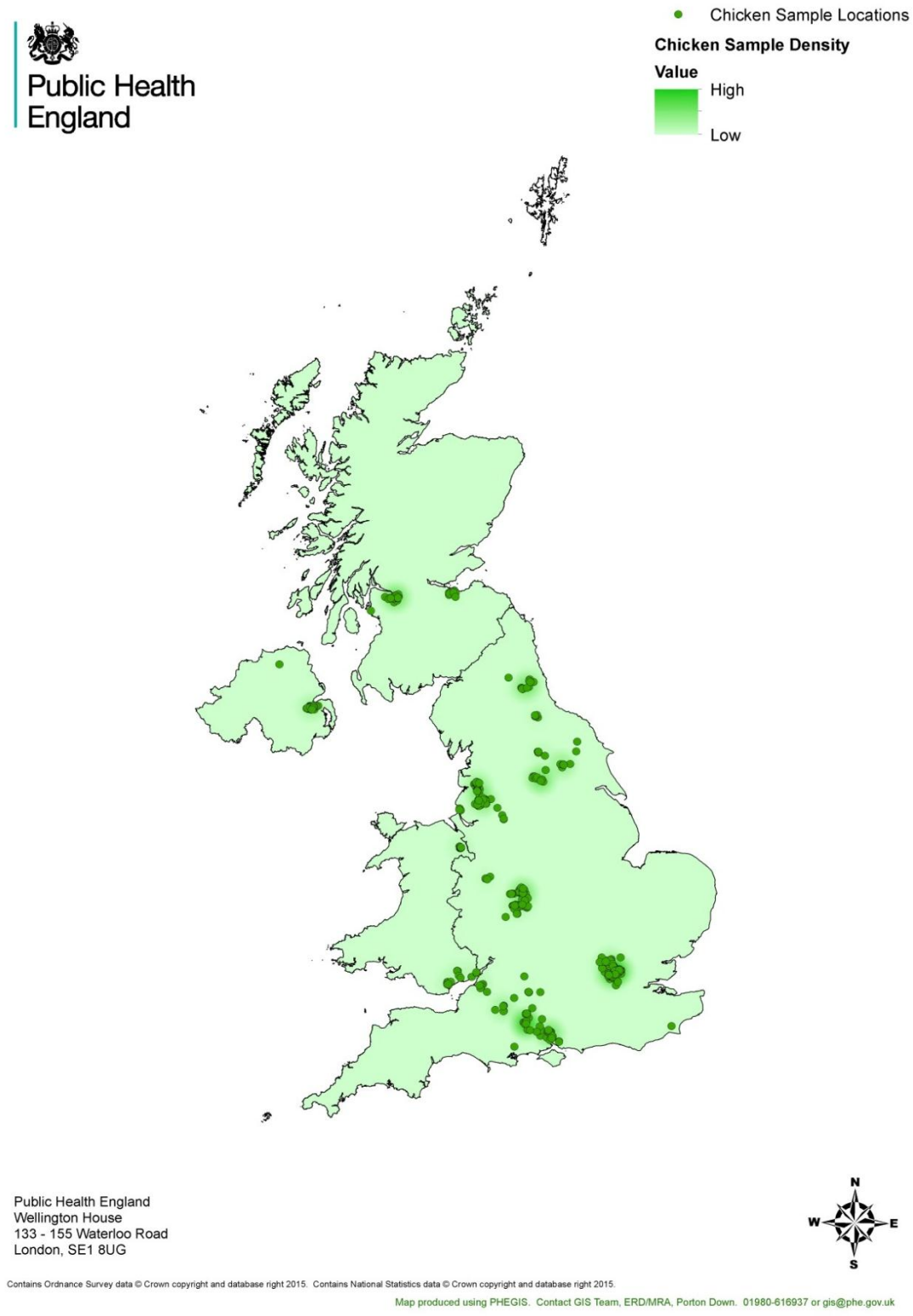

Figure 1. Geographical distribution of samples collected for the survey.

Chicken skin samples and outer packaging samples were tested to determine the number of cfu of *Campylobacter* spp. by the direct enumeration test recommended by ISO. The full dataset for this survey is presented in Appendix III.

3.1 Numbers of campylobacters in chicken skin and outer packaging samples from whole fresh UK produced chicken.

Campylobacters were detected in the majority of chicken skin samples (73.3 % (95% CI 72.0 % to 74.7 %)) and 19.4 % (95% CI 18.2 % to 20.7 %) of the chicken skin samples (n = 4011 tested) had counts above 1000 cfu/g chicken skin. The highest count detected was 640,000 cfu of *Campylobacter* per g chicken skin. In packaging swab samples (n = 4005), 6.8 % (95% CI 6.0 % to 7.6 %) of samples had *Campylobacter* spp. isolated, but mostly at low level.

3.1.1 Campylobacters in chicken skin samples in relation to retailer.

One of the retailers (ASDA) had a higher proportion of chickens with *Campylobacter* spp. levels at >1000 cfu/g compared to the average of 19.4 % for all retailers while another retailer (Tesco) had a lower proportion of samples with >1000 cfu than the average (Table 1). It should be noted that only a relatively small number of samples were tested from certain retailers (e.g. Waitrose). For such retailers this lowers the precision in estimating the percentage contaminated and limits the extent to which conclusive inferences may be drawn.

Table 1. Numbers of *Campylobacter* spp. in skin samples from fresh whole UK produced 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)**
Asda (662)	129	19.5 (16.5-22.7)	112	16.9 (14.1-20.0)	223	33.7 (30.1-37.4)	198	29.9 (26.4-33.6)
Co-op (378)	79	20.8 (16.8-25.2)	90	23.7 (19.5-28.3)	135	35.5 (30.7-40.6)	74	19.5 (15.6-23.8)
M&S (130)	41	31.5 (23.7-40.3)	32	24.6 (17.5-33.0)	34	26.2 (18.8-34.6)	23	17.7 (11.6-25.4)
Morrisons (349)	85	24.4 (19.9-29.2)	62	17.8 (13.9-22.2)	125	35.8 (30.8-41.1)	77	22.1 (17.8-26.8)
Sainsbury's (557)	167	30.0 (26.2-34.0)	124	22.3 (18.9 – 26.0)	177	31.8 (27.9-35.8)	89	16.0 (13.0-19.3)
Tesco (1235)	408	33.0 (30.4-35.7)	320	25.9 (23.5-28.5)	348	28.2 (25.7-30.8)	159	12.9 (11.1-14.9)
Waitrose (111)	28	25.2 (17.5-34.4)	30	27.0 (19.0-36.3)	34	30.6 (22.2-40.1)	19	17.1 (10.6-25.4)
Others[#] (589)	132	22.4 (19.1-26.0)	132	22.4 (19.1-26.0)	184	31.2 (27.5-35.2)	141	23.9 (20.6-27.6)
Total (4011)	1069	26.7 (25.3-28.0)	902	22.5 (21.2-23.8)	1260	31.4 (30.0-32.9)	780	19.4 (18.2-20.7)

*n = Number of samples

[#]Others included supermarkets with lower market shares (Kantar 2010) and independents e.g. Lidl, Aldi, Iceland, convenience stores, butchers.

3.1.2 Numbers in outer packaging samples in relation to retailer.

The prevalence and level of contamination found in the outer packaging samples was low. A significantly lower proportion of packaging positive for campylobacters was found from Tesco and significantly higher proportions of contaminated packaging was identified for Asda and Morrisons, compared to the overall average of 6.8 % (Table 2).

Table 2. Numbers of *Campylobacter* spp. in outer packaging samples from fresh whole UK produced chicken in relation to retailer.

Retailer (n*)	cfu of *Campylobacter* spp. per outer packaging swab							
	<10		10-99		100-1000		>1000	
	n	% (95 % CI)	n	% (95 % CI)	n	% (95 % CI)	n	% (95 % CI)
Asda (662)	579	87.5 (84.7-89.9)	56	8.5 (6.5-10.8)	25	3.8 (2.5-5.5)	2	0.3 (0.0-1.1)
Co-op (380)	361	95.0 (92.3-97.0)	15	4.0 (2.2- 6.4)	4	1.1 (0.3-2.7)	0	0.0 (0.0-1.0)
M&S (130)	126	96.9 (92.3- 99.2)	4	3.1 (0.8- 7.7)	0	0.0 (0.0-2.8)	0	0.0 (0.0-2.8)
Morrisons (349)	309	88.5 (84.7-91.7)	32	9.2 (6.4-12.7)	7	2.0 (0.8-4.1)	1	0.3 (0.0-1.6)
Sainsbury's (550)	524	95.3 (93.2-96.9)	22	4.0 (2.5-6.0)	3	0.6 (0.1-1.6)	1	0.2 (0.0-1.0)
Tesco (1235)	1184	95.9 (94.6-96.9)	37	3.0 (2.1-4.1)	14	1.1 (0.6- 1.9)	0	0.0 (0.0-0.3)
Waitrose (111)	102	91.9 (85.2-96.3)	8	7.2 (3.2-13.7)	0	0.0 (0.0-3.3)	1	0.9 (0.0-4.9)
Others[#] (588)	548	93.2 (90.9-95.1)	35	6.0 (4.2-8.2)	5	0.9 (0.3-2.0)	0	0.0 (0.0-0.6)

*n = Number of samples

[#]Others included supermarkets with lower market shares (Kantar 2010) and independents e.g. Lidl, Aldi, Iceland, convenience stores, independents, butchers.

3.1.3 Numbers of campylobacters 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. Considerable fewer samples from chickens reared using free range or organic production methods were examined reflecting 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 in contamination rates between these. The chickens reared using organic production methods appeared to have a higher proportion that were highly contaminated. However, as only 28 were tested in this survey there is limited statistical power to make any conclusive statement as to whether these birds are more or less highly contaminated than standard birds, or whether the observed result is due to sampling variation.

Table 3. Numbers of *Campylobacter* spp. in skin samples from fresh whole UK produced 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 (3689)	994	26.9 (25.5-28.4)	805	21.8 (20.5-23.3)	1178	31.9 (30.4-33.4)	712	19.3 (18.0-20.6)
Free Range (284)	59	20.7 (16.2-25.9)	90	31.6 (26.2-37.3)	79	27.7 (22.6-33.3)	56	19.7 (15.2-24.7)
Organic (28)	13	46.4 (26.6-66.6)	5	17.9 (4.4-34.9)	2	7.7 (0.9-25.1)	8	28.6 (14.3-51.8)
Not Provided# (10)	3	30.0 (6.7-65.2)	2	20.0 (2.5-55.6)	1	10.0 (0.3-44.5)	4	40.0 (12.1-73.8

*n = Number of samples;
# chicken type not provided on label or retailer not able to verify chicken rearing type

3.1.4 Numbers of campylobacters in chicken skin samples in relation to chicken processor approval number.

There were clear, statistically significant differences ($p < 0.0001$), in the distribution of contamination of chicken with *Campylobacter* spp. between the different processor approval numbers (i.e. slaughter house premises; Table 4).

Table 4. Numbers of *Campylobacter* spp. in skin samples from fresh whole UK produced chicken in relation to processor approval number. Approval numbers listed represent those where ≥ 50 chickens were tested in the study.

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)**
1100 (346)	96	27.8 (23.1-32.8)	83	24.0 (19.6-28.8)	96	27.8 (23.1-32.8)	71	20.5 (16.4-25.2)
2037 (473)	124	26.2 (22.3-30.4)	115	24.3 (20.5-28.4)	154	32.6 (28.4-37.0)	80	16.9 (13.7-20.6)
3007 (418)	73	17.5 (14.0-21.5)	63	15.1 (11.8-18.9)	158	37.8 (33.1-42.6)	124	29.7 (25.3-34.3)
3011 (339)	135	39.8 (34.6-45.3)	90	26.6 (21.9-31.6)	82	24.2 (19.7-29.1)	32	9.4 (6.6-13.1)
4014 (275)	67	24.4 (19.4-29.9)	65	23.6 (18.7-29.1)	79	28.7 (23.5-34.5)	64	23.3 (18.4-28.7)
5008 (306)	56	18.3 (14.1-23.1)	72	23.5 (18.9-28.7)	115	37.6 (32.1-43.3)	63	20.6 (16.2-25.6)
5011 (410)	137	33.4 (28.9-38.2)	92	22.4 (18.5-26.8)	122	29.8 (25.4-34.4)	59	14.4 (11.1-18.2)
5464 (272)	45	16.5 (12.3-21.5)	40	14.7 (10.7-19.5)	116	42.7 (36.7-48.8)	71	26.1 (21.0-31.8)
7009 (104)	45	43.3 (33.6-53.4)	26	25.0 (17.0-34.5)	22	21.2 (13.8-30.3)	11	10.6 (5.4-18.1)
8005 (430)	128	29.8 (25.5-34.3)	106	24.7 (20.7-29.0)	133	30.9 (26.6-35.5)	63	14.7 (11.5-18.4)
9502 (272)	67	24.6 (19.6-30.2)	91	33.5 (27.9-39.4)	77	28.3 (23.0-34.1)	37	13.6 (9.8-18.3)
9509 (77)	18	23.4 (14.5-34.4)	20	26.0 (16.6-37.2)	27	35.1 (24.5-46.8)	12	15.6 (8.3-25.6)
Other code[#] (209)	53	25.4 (19.6-31.8)	31	14.8 (10.3-20.4)	61	29.2 (23.1-35.9)	64	30.6 (24.5-37.4)
Not Available§ (80)	25	31.3 (21.4-42.6)	8	10.0 (4.4-18.8)	18	22.5 (13.9-33.2)	29	36.3 (25.8-47.8)

*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.

The percentage of chickens with >1000 cfu/g ranged from 9.4 % (processor approval number 3011) to 29.6 % (processor approval number 3007). The "other" group of approval numbers that had less than 50 chickens examined in the survey also had a high proportion of chickens with >1000 cfu/g. The variation in the percentage of samples with high levels of *Campylobacter* spp. contamination for different processor approval numbers can be seen in Figure 2.

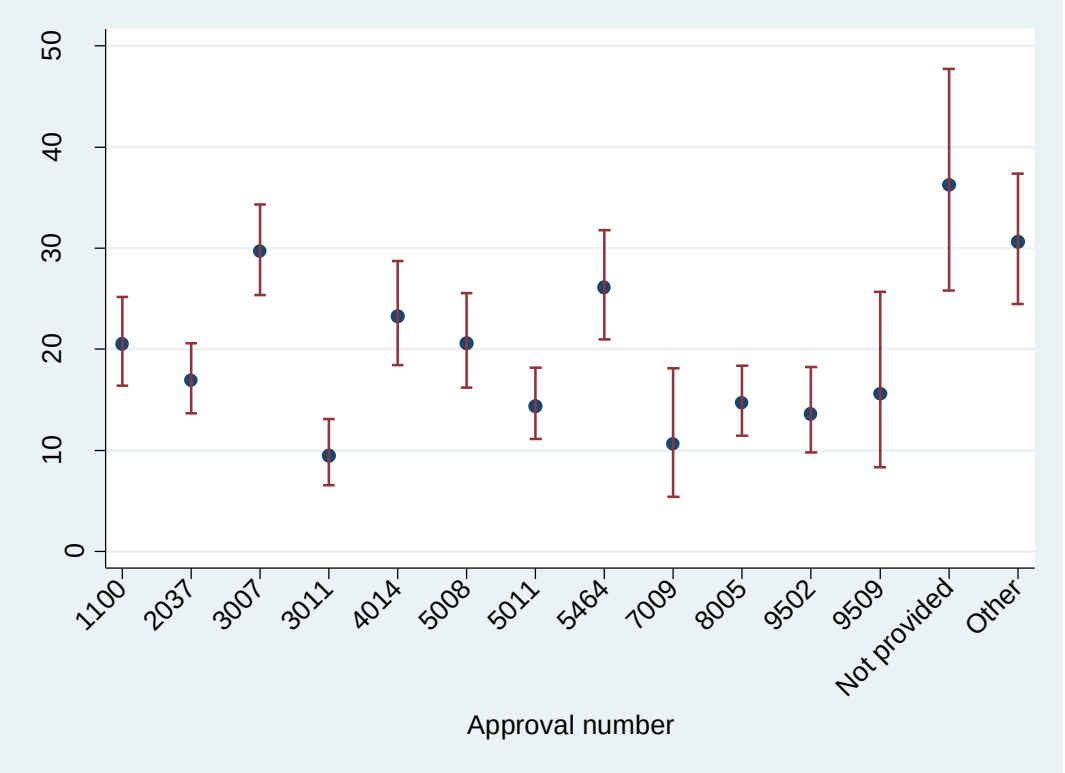

Figure 2. Percent of samples with high levels of campylobacters by processor approval number (the bars are the 95 % CI for the mean plotted for each processor).

3.1.5 Numbers of campylobacters in chicken skin samples in relation to season

Significant variation in levels was detected for the different season months. A higher proportion of chickens were identified to have a high level of *Campylobacter* spp. during summer compared to winter months (Table 5).

Table 5. Numbers of *Campylobacter* spp. in skin samples from fresh whole UK produced chicken in relation to season.

Season (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)**
Spring Mar/Apr/May (992)	374	37.7 (34.7-40.8)	193	19.5 (17.0-22.1)	261	26.3 (23.6-29.2)	164	16.5 (14.3-19.0)
Summer Jun/Jul/Aug (1156)	186	16.1 (14.0-18.3)	278	24.1 (21.6-26.6)	444	38.4 (35.6-41.3)	248	21.5 (19.1-23.9)
Autumn Sep/Oct/Nov (1001)	237	23.7 (21.1-26.4)	258	25.8 (23.1-28.6)	311	31.1 (28.2-34.0)	195	19.5 (17.1-22.1)
Winter Dec/Jan/Feb (862)	272	31.6 (28.5-34.8)	173	20.1 (17.4-22.9)	244	28.3 (25.3-31.4)	173	20.1 (17.4-22.9)

*n = Number of samples

3.1.6 Numbers of campylobacters 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 permitted the separation of the data. This allowed for analysis to determine whether size, which is generally 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. Numbers of *Campylobacter* spp. in skin samples from fresh whole UK produced 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 (1142)	430	37.0 (34.8-40.5)	261	22.9 (20.5-25.4)	288	25.2 (22.7-27.9)	163	14.3 (12.3-16.5)
Medium 1400-1750 g (1796)	444	24.7 (22.7-26.8)	387	21.6 (19.7-23.5)	604	33.6 (31.5-35.9)	361	20.1 (18.3-22.0)
Large >1750 g (1021)	181	17.7 (15.4-20.2)	245	24.0 (21.4-26.7)	358	35.1 (32.1-38.1)	237	23.2 (20.7-25.9)
Not recorded (51)	14	27.5 (15.9-41.7)	9	17.7 (8.4- 30.9)	10	19.6 (9.8-33.1)	18	35.3 (22.4-49.9)

*n = Number of samples

3.1.7 Numbers of campylobacters in chicken skin samples in relation to days of shelf-life remaining

Chickens were tested with up to 9 days shelf-life remaining (Table 7). The most frequent number of days of shelf-life remaining when the chickens were tested was five days. There does not appear to be an association between high level contamination and the length of shelf-life remaining in days, i.e. with those birds that are closer to their production date.

Table 7. Numbers of *Campylobacter* spp. in skin samples from fresh whole UK produced chicken in relation to days of remaining shelf-life.

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 (270)	79	29.3 (23.9-35.1)	57	21.1 (16.4-26.5)	77	28.5 (23.2-34.3)	57	21.1 (16.4-26.5)
2 (404)	126	31.2 (26.7-36.0)	79	19.6 (15.8-23.8)	120	29.70 (25.3-34.4)	79	19.6 (15.8-23.8)
3 (646)	175	27.1 (23.7-30.7)	154	23.8 (20.6-27.3)	200	30.96 (27.4-34.7)	117	18.1 (15.2-21.3)
4 (821)	206	25.1 (22.2-28.2)	208	25.3 (22.4-28.5)	257	31.30 (28.1-34.6)	150	18.3 (15.7-21.1)
5 (868)	251	28.9 (25.9-32.1)	193	22.2 (19.5-25.2)	258	29.7 (26.7-32.9)	166	19.1 (16.6-21.9)
6 (623)	141	22.6 (19.4-26.1)	139	22.3 (19.1-25.8)	225	36.1 (32.3-40.0)	118	19.0 (15.9-22.2)
7-9 (318)	77	19.7 (15.9-24.0)	149	38.2 (33.4-43.2)	90	23.1 (19.0-27.6)	74	19.0 (15.2-23.2)
Not available (61)	14	23.0 (13.2-35.5)	11	18.0 (9.4-30.0)	17	27.9 (17.2-40.8)	19	31.2 (19.9-44.3)

*n = Number of samples

3.1.8 Other sample details

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

3.2 Logistic regression

Analysis of the cfu of *Campylobacter* per g of chicken skin did not detect noticeable confounding factors (Table 8). With the exception of remaining shelf-life, the multivariable logistic regression model provided extremely similar estimates of odds ratios to those obtained when each variable was considered in isolation in the single variable logistic regression analysis. This indicated that no strong confounding was occurring in the results presented. The variation in the percentage contamination in chickens from the different retailers could not be explained on the basis of confounding, and as such is likely to represent genuine variation between the retailers.

It was decided to focus the analysis around differences between retailers, in line with the interim publications of the accumulated study data produced by the FSA. 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 processor has a bearing on contamination rate and this will be manifested as variation in the contamination rate between retailers. The study was designed to reflect retailer market share. 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.

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	
Asda	1.75 (1.29-2.38)		1.89 (1.39-2.57)	
M&S	0.88 (0.53-1.48)		0.94 (0.56-1.58)	
Morrisons	1.16 (0.81-1.66)		1.20 (0.84-1.73)	
Others	1.29 (0.94-1.77)		1.22 (0.87-1.72)	
Sainsbury's	0.78 (0.56-1.10)		0.82 (0.57-1.17)	
Tesco	0.61 (0.45-0.82)		0.61 (0.45-0.84)	
Waitrose	0.85 (0.49-1.48)		1.02 (0.57-1.81)	
Chicken type		0.5		0.4
Standard	Reference		Reference	
Free Range	1.03 (0.75-1.39)		1.08 (0.78-1.50)	
Organic	1.67 (0.73-3.81)		1.77 (0.75-4.16)	
Season		0.03		0.06
Spring	0.79 (0.62-1.00)		0.81 (0.63-1.04)	
Summer	1.09 (0.87-1.35)		1.11 (0.88-1.39)	
Autumn	0.96 (0.77-1.21)		0.98 (0.77-1.25)	
Winter	Reference		Reference	
Remaining shelf-life		0.6		0.01
Per additional day	1.01 (0.97-1.06)		1.07 (1.01-1.12)	
Weight		<0.001		<0.001
Small <1400 g	Reference		Reference	
Medium 1400-1750 g	1.51 (1.23-1.85)		1.31 (1.05-1.62)	
Large >1750 g	1.81 (1.45-2.25)		1.71 (1.36-2.15)	

Several variables that customers may identify at the point of purchase were included in the multivariable analysis (Table 8).

The larger chickens were found to be more likely to have high counts of *Campylobacter* spp. per g skin in the logistic regression model and this was investigated further. For the large, and to some extent, the medium chickens in this survey, there was an absence of the expected seasonal pattern of increasing contamination in the summer and a reduced contamination in the winter and spring months. Including this interaction into the multivariable logistic regression analysis a significant interaction remains after adjustment for any confounding effects of the other variables in the model (Table 9).

Table 9. Percentage of chickens with contamination levels >1000 cfu of *Campylobacter* spp. per g chicken skin within each weight and season category.

Season	Weight category			
	Small (<1400 g)	**Medium (1400 - 1750 g)**	**Large (> 1750 g)**	**Total**
Spring	8.9 %	17.2 %	26.2 %	16.2%
Summer	18.7 %	22.3 %	22.6 %	21.3%
Autumn	16.7 %	21.0 %	18.9 %	19.4%
Winter	12.9 %	19.6 %	26.5 %	19.7%
Total	14.3 %	20.1 %	23.2 %	19.2%

Additional analysis was performed by including variables not normally available to consumers, but nonetheless may relate to the level of *Campylobacter* spp. contamination.

While the protocol stipulated to test a 25 g neck-skin sample not all chickens had sufficient neck-skin available for analysis so for those, the sample was supplemented with breast-skin to ensure 25 g of skin was tested. To assess this, a categorical variable of '0 g', 'up to 5 g', and '5 or more g' of breast skin in the sample was included into the multivariable model above. The estimated odds ratio and 95 % confidence intervals of the 'up to 5 g' of breast skin compared to those samples with no breast skin was 0.92 (95 % CI 0.68 to 1.23) indicating a non-significant ($p = 0.6$) reduction in high level contamination for this category. For samples with 5 or more grams of breast skin, the estimated odds ratio compared to those with no breast skin was 0.40 (95 % CI = 0.30 to 0.53), indicating a significant reduction ($p < 0.001$) in high level contamination in this category. The odds ratios of the other variables in the multivariable model were similar to those obtained in the multivariable logistic regression model that did not contain the breast skin categories as a predictor. This indicates that while the proportion of breast skin does influence the contamination rate, it does not confound the other associations identified including the retailer association. One consequence of this finding is that, had there been sufficient neck skin present in all chickens tested, the contamination rate may have been greater than that observed in this survey.

While there was no obvious scientific reason to assume the price of a chicken would be associated with the level of contamination, a strong association between the weight of the chicken and its purchase price was identified (Appendix V). In addition there was a strong association between the type of chicken and purchase price. The mean purchase price for free range, organic, and standard chickens were £7.77, £9.31 and £4.65, respectively. There was a weak association between the cost of the chicken and the proportion contaminated with > 1000 cfu of *Campylobacter* per g skin. The estimated odds ratio for high level contamination was 1.05 per £1 increase in price (95 % CI 1.00 to 1.11, $p=0.04$). However, when cost was included in the logistic regression model it was no longer statistically significant ($p=0.14$), indicating that the weight of chicken rather than its price was the more important predictor of high level *Campylobacter* spp. contamination.

According to the protocol (FSA 2014) sample box temperatures during transport and at sample delivery were considered when assigning a satisfactory or unsatisfactory sample receipt. Only samples assigned with a satisfactory sample receipt were accepted for testing. When assigning a satisfactory sample receipt, the temperature of the sample when collected and transport time was considered. It is acknowledged that *Campylobacter* spp. may survive on chicken skin better at fridge temperatures than at temperatures of between 25 and 42 °C but there is little evidence to suggest significant differences in die-off between 5 and 15 °C (Solow *et al*., 2003; Davis and Conner 2007). Viability of the *Campylobacter* bacteria declines when exposed to freezing temperatures. It is also worth noting that, unlike many other bacteria, *Campylobacter* spp. are unable to grow and multiply at the temperatures normally used for storing raw meat products and their temperature range for growth is 30 – 45 °C, with an optimum of 42 °C. In this survey, for those chickens with a temperature at collection of greater than 8 °C there was a non-significant increase ($p = 0.9$) in high level contamination with an estimated odds ratio of 1.04 (95 % CI = 0.52 to 2.07). A similar non-significant increase ($p = 0.8$) in high level contamination with an estimated odds ratio of 1.06 (95% CI = 0.70 to 1.62) was seen with temperature on receipt greater than 8°C.

In relation to the outer packaging, the results from the multivariable regression are very similar when the variables are considered in isolation with the exception of remaining shelf life. This indicates that there is no strong confounding effect of the results obtained when each factor is analysed alone (Table 10). Additional analysis of the counts of *Campylobacter* spp. found in the outer packaging samples demonstrated no significant interactions between factors in the multivariable model.

Table 10. Estimated odds ratios from single variable analysis and multivariable logistic regression models of the ≥10 cfu of *Campylobacter* spp. found on outer packaging samples.

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	
Asda	2.72 (1.62-4.56)		2.65 (1.58-4.46)	
M&S	0.60 (0.20-1.81)		0.58 (0.19-1.75)	
Morrisons	2.46 (1.39-4.34)		2.53 (1.43-4.50)	
Others	1.39 (0.79-2.43)		1.43 (0.80-2.57)	
Sainsbury's	0.94 (0.51-1.73)		0.89 (0.48-1.66)	
Tesco	0.82 (0.47-1.40)		0.81 (0.46-1.40)	
Waitrose	1.68 (0.73-3.82)		1.72 (0.73-4.05)	
Chicken type		0.008		0.02
Standard	Reference		Reference	
Free Range	0.43 (0.22-0.85)		0.47 (0.24-0.94)	
Organic	2.41 (0.82-7.04)		2.18 (0.72-6.59)	
Season		0.02		0.02
Winter	Reference		Reference	
Spring	0.92 (0.62-1.37)		0.95 (0.63-1.44)	
Summer	1.43 (1.00-2.05)		1.49 (1.03-2.15)	
Autumn	1.44 (1.00-2.09)		1.41 (0.97-2.06)	
Remaining shelf-life		0.043		0.48
Per additional day	0.93 (0.86-0.997)		0.97 (0.90-1.05)	
Weight		0.013		0.01
Small <1400 g	Reference		Reference	
Medium 1400-1750 g	1.21 (0.88-1.66)		1.02 (0.73-1.43)	
Large >1750 g	1.64 (1.17-2.29)		1.57 (1.11-2.22)	

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

For the majority of chicken skin samples (76.6 %) from which isolates were submitted for speciation, *C. jejuni* alone was identified while *C. coli* alone was identified in 13.9 % of samples (Table 11). Both these species were found in 4.2 % of samples while for the remaining positive samples, *C. jejuni* or *C. coli* were not detected or no speciation test was performed due to loss of isolate viability.

Table 11. *Campylobacter* spp. isolates from skin samples of fresh whole UK produced chicken.

	No. of samples	**% of samples**
***C. jejuni* only**	2253	76.6
***C. coli* only**	409	13.9
Mixed *C. jejuni* and *C. coli*	124	4.2
***C. jejuni* (including mixed)**	2377	80.8
***C. coli* (including mixed)**	533	18.1
Other *Campylobacter spp.*	156	5.6

The proportion of samples from which *C. jejuni* was isolated was higher in the spring months compared to the summer months. Conversely, *C. coli* was more frequently isolated during the summer months, which was found to be significant (Table 12).

Table 12. *C. jejuni* and *C. coli* isolates in fresh whole UK produced chicken in relation to season.

	Season[a] % of samples with species (no. of samples)			
	Spring (n = 618)	**Summer** (n = 970)	**Autumn** (n = 764)	**Winter** (n = 590)
***C. jejuni* only**	85.4 (528)	69.8 (677)	78.4 (599)	76.1 (449)
***C. coli* only**	8.1 (50)	19.9 (193)	16.1 (123)	7.3 (43)
Mixed *C. jejuni* & *C. coli*	1.6 (10)	8.2 (80)	3.4 (26)	1.4 (8)

[a]For the purposes of this report, Spring is defined as March, April and May, Summer as June, July and August, Autumn as September, October and November, and Winter as December, January and February.

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; $p<0.0001$ Fisher's exact). However, further data would be required to ascertain this observation as only a small number of organic birds were tested. Nonetheless, the probability of observing 8 of the 15 tested as positive for *C. coli* is very small if the true proportion of positives is 0.15 ($p=0.0006$ exact binomial test) (Table 13).

Table 13. *C. jejuni* and *C. coli* isolates from fresh whole UK produced chicken in relation bird rearing regime.

	Chicken reared as: % of samples with Campylobacter species (no. of samples)		
	Standard rearing (no access to range) (n = 2549)	**Free range** (n = 225)	**Organic** (n = 15)
***C. jejuni* only**	83.0 (2116)	54.7 (123)	46.7 (7)
***C. coli* only**	12.8 (325)	33.8 (76)	53.3 (8)
C. jejuni* & *C. coli	4.2 (108)	7.1 (16)	0 (0)

For some processor approval numbers, a slightly higher proportion of *C. coli* appeared to be isolated than the average for all approval numbers (Table 14). While some of the observed variation may be due to the chicken rearing regime, after allowing for this there remains a statistically significant association ($p<0.0001$ multinomial logistic regression). It is possible that there could be an association between *Campylobacter* strain types and retailer and/or processors.

Table 14. *C. jejuni* and *C. coli* isolates from fresh whole UK produced chicken in relation to approval number.

Approval code	***C. jejuni* only**		***C. coli* only**		***C. jejuni* and *C. coli***		**Other *Campylobacter spp.***	
	%	**No. of samples**	**%**	**No. of samples**	**%**	**No. of samples**	**%**	**No. of samples**
1100	83.6	209	10.8	27	3.6	9	2.0	5
2037	81.7	285	4.6	16	3.7	13	10.0	35
3007	90.7	313	2.6	9	2.3	8	4.3	15
3011	74.5	152	15.2	31	6.9	14	3.4	7
4014	77.4	161	13.0	27	1.9	4	7.7	16
5008	68.0	170	21.2	53	4.0	10	6.8	17
5011	74.7	204	15.4	42	5.9	16	4.0	11
5464	70.0	159	18.1	41	5.3	12	6.6	15
7009	84.7	50	6.8	4	1.7	1	6.8	4
8005	75.5	228	14.9	45	5.3	16	4.3	13
9502	73.2	115	20.4	32	3.2	5	3.2	5
9509	77.6	38	18.4	9	4.1	2	0.0	0
Other codes	68.6	83	22.3	27	3.3	4	5.8	7
Code not provided	76.5	26	17.6	6	2.9	1	2.9	1

The majority of *Campylobacter* strains isolated from the outer packaging samples were *C. jejuni*. The proportions of *C. jejuni* and *C. coli* isolated from outer packaging samples was similar to that found in chicken neck-skin samples (Table 15).

Table 15. *C. jejuni* and *C. coli* isolates from outer packaging of fresh whole UK produced chicken.

	No. of samples	**% of total samples speciated** (n = 272)
***C. jejuni* only**	206	75.7
***C. coli* only**	34	12.5
Mixed *C. jejuni* and *C. coli*	14	5.1
Other *Campylobacter* spp.	18	6.6
***C. jejuni* (including mixed)**	220	80.9
***C. coli* (including mixed)**	48	17.6

Comparison of isolates from 235 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 16). However, on 16 occasions (7 %) a different *Campylobacter* species was detected in the two samples that had been derived from the same chicken pack.

Table 16. *C. jejuni* and *C. coli* species from outer packaging and corresponding skin sample of fresh whole UK produced chicken.

		***Campylobacter* species detected in swab**		
		***C. jejuni* only**	***C. coli* only**	***C. jejuni* and *C. coli***
***Campylobacter* species detected in skin sample**	***C. jejuni* only**	182	7	7
	***C. coli* only**	9	23	5
	C. jejuni* and *C. coli	2	2	2

4. DISCUSSION

In this survey the estimated prevalence of *Campylobacter* spp. in fresh whole UK produced chicken at retail was 73.3 % while 19.4 % of samples had >1000 cfu per g neck skin. In a survey undertaken in 2007-2008 (FSA 2009), 15.5 % of whole fresh chickens had >1000 cfu per g. The much lower numbers of whole fresh UK produced chickens tested in the earlier survey and the use of different testing methods, however, hinder a direct comparison.

This work has for the first time provided numbers of campylobacters on outer packaging of retail chicken packs. In 6.8 % of samples *Campylobacter* spp. were detected from the outer-packaging and while this was mostly at low levels, 1.6 % of samples had between 100 and 4500 *Campylobacter* cfu per swab. The presence of *Campylobacter* spp. on the outer packaging of chicken packs is concerning 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.

There were significant differences in the proportion of highly contaminated chickens between some major retailers. Compared against the industry average, Tesco had the lowest proportion of highly contaminated chickens, while ASDA had the highest. Such differences could relate to a number of factors including chicken rearing factors (e.g. farm biosecurity levels and whether they were reared with access to range), processing plant, age of bird at slaughter, season and shelf-life assigned. Accurate details were not available for all of these factors for all chickens tested. Nevertheless statistical analysis demonstrated that neither rearing regime, bird 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 campylobacters found on whole fresh chicken. However, the strong relationship between retailer and approval number precludes an investigation of approval number in the logistic regression analyses. Additionally, approval code is unlikely to feature in consumer purchasing decisions.

While there was no evidence that free-range or organic chickens were more or less highly contaminated than standard birds, this finding should be treated with caution as low numbers of free-range and organic chickens were examined. Their corresponding confidence intervals were wide and would therefore only be able to verify very large differences.

The data suggested that a higher proportion of chickens had > 1000 cfu of campylobacters per g of skin during the summer compared to the winter months. This was, however, only prominent for lower weight birds and data from further 12 month testing periods would be needed to ascertain the significance of this observation. It is possible that the heavier birds reflect thinned batches that are known to be more likely to be contaminated. The prevalence of *Campylobacter* spp. in retail chicken, as determined by the enrichment-based detection (also known as the presence/ absence test) method, has previously been associated with the time of year sampled (Meldrum 2005, CLASSP Project Team 2010, Hutchinson *et al*. 2006).

Variation in levels of *Campylobacter* isolated across the six laboratories was identified within this study. A review of the External Quality Assurance data and Internal Quality Control data collected throughout the life of the project was performed (Appendix IV). This data showed consistently satisfactory test results across the six laboratory sites. It is possible that other underlying factors not recorded in the study including the precise chicken type and associated variation in *Campylobacter* contamination between regional outlets could manifest itself as variation between testing laboratories. Consumer demands are likely to vary between regions and the types of chicken sold are likely to reflect such demands. It was also apparent that chickens sold by some retailers differed in terms of processor approval number depending on which region they were sold in. This phenomenon will continue to be monitored and reviewed in the FSA project FS102121, which extends the survey presented here for up to a further three years.

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, the data could suggest that these two species have a similar ability to contaminate and persist on outer packaging.

From the majority of chicken skin samples (76.6 %) *C. jejuni* was isolated while *C. coli* was identified in 13.9 % of samples. From a small proportion of samples (70) campylobacters were isolated that were not classified as *C. jejuni* or *C. coli* and consideration of subjecting these isolates for further characterisation using whole genome sequencing is being made. In another FSA commissioned survey carried out in 2007 and 2008 (FSA 2009) looking at campylobacters on raw chickens at UK retail, the proportion of chickens from which *C. jejuni* was isolated appeared to be lower than in the current study. Conversely, the present survey identified *C. coli* in a lower proportion of samples than found in the 2007/2008 FSA survey. In the 2007/2008 survey campylobacters were detected in 825 samples of fresh whole UK chickens. Of the 2007/2008 survey samples (635) where strains were sent for speciation *C. jejuni* was identified in 43 % while *C. coli* was identified in 35 % (and both species were detected in 22 % of samples). It is possible that this finding may relate to differences in the method of detection used in the two surveys. While this survey applied direct enumeration only, the 2007/2008 survey isolates were obtained using an enrichment method. 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 over 2000 raw whole chickens were tested using the enrichment culture method for campylobacters. Of the strains isolated from that study 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.

Recent slaughter house survey data for campylobacters on chicken carcasses tested just after slaughter (and before being on retail sale) undertaken by the APHA found a decrease in the proportion of contaminated carcases from approximately 79 % in 2012-13 to approximately 72 % in 2014-15 (FSA 2015c). This could suggest a recent

downward trend that may also manifest itself in retails chickens but continued monitoring would be needed to verify if this could be the case.

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 number of human *Campylobacter* cases reported in the UK showed an increase between 2003 to 2012 but from 2013 there may be evidence of a decline and surveillance will help ascertain if this is a continuing trend (Table 17)

Table 17. Human cases of campylobacteriosis from 2003 to 2014 (PHE 2015)

	Number of human campylobacteriosis cases	
Year	**in England and Wales**	**in the UK**
2003	46291	52126
2004	44577	50388
2005	46735	52686
2006	46853	52134
2007	51982	57815
2008	50006	55609
2009	57784	65043
2010	63080	70298
2011	64725	72150
2012	65044	72560
2013*	59041	66465
2014*	60604	Not available yet

*Provisional number of cases (PHE 2015)

5. CONCLUSIONS

The overall prevalence of *Campylobacter* spp. in the fresh chicken at retail in the UK was found to be 73.3 %. A considerable proportion (19.4 %) of samples had > 1000 cfu per g chicken skin. The proportion of samples with > 1000 cfu per g chicken skin ranged from 12.9 to 29.9 % between retailer groups. 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* 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 processor 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 %.

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.

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 and both species were found in 4.2 % of samples. *C. coli* was more frequently isolated in the summer compared to winter and spring months. 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.

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.

The results reported here represent the first year in an ongoing survey. The second year of sampling began in July 2015 and adopted a substantially similar design. The most material difference relates to the priority given to sampling retailers. Based on new market share data, two additional retailers (Aldi and Lidl) have been deemed large enough to merit a fixed quota of samples: these will be added to the seven named retailers in the current report. To enable more precise comparison of the nine named retailers, each will be allocated the same number of samples: 400. Appropriate re-weighting will be required to recover the UK average prevalence of campylobacter, with only a minor effect on the precision at this level.

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APPENDIX I: Number of chickens sampled versus planned sampling for the duration of the project

Area of sampling	Planned for project	Actual for period
England	3355	3365
Wales	193	196
Scotland	336	335
Northern Ireland	116	116
Total	4000	4012

APPENDIX II: Pilot study (embedded Word document)

PHEAFBI Final Pilot Project Report.docx

APPENDIX III: Detailed survey dataset (embedded excel document)

Final report
FS241044 raw data.x

APPENDIX IV: Quality Control Data

To maintain the quality assurance in their testing, each laboratory involved in the study participates in external quality assurance schemes for the method used. Samples for this testing are supplied by an external body and results are submitted back to the provider for analysis allowing an independent means of reviewing the testing capability of the laboratory. Table IVa shows the results for each laboratory for the PHE Food and Environment Proficiency Testing Unit External Quality Assurance Standard Scheme and Table IVb shows results from the FSA EQA Scheme for *Campylobacter* spp. enumeration, which provides interpretation of results through absolute z scores.

Table IVa. Results for the six laboratories for the PHE Food and Environment Proficiency Testing Unit External Quality Assurance Standard Scheme

Distribution Reference / Date	Sample Number	Sample Contents	Expected Result	Laboratory Result Reported					
				1	2	3	4	5	6
258 July 2014	SO553	No *Campylobacter* spp.	Enrichment: Not Detected in 25 g	Not Detected in 25 g	Not Detected in 25 g	Not Detected in 25 g	Not Detected in 25 g	Not Detected in 25 g	Not Detected in 25 g
			Enumeration: <10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g	<20 cfu/g	<10 cfu/g	<10 cfu/g
	SO554	*Campylobacter jejuni* (wild type strain)	Enrichment: Detected in 25 g	Detected in 25 g	Detected in 25 g	Detected in 25 g	Detected in 25 g	Detected in 25 g	Detected in 25 g
			Enumeration: 10 - 280 cfu/g	30 cfu/g	280 cfu/g	10 cfu/g	100 cfu/g	40 cfu/g	13 cfu/g
264 January 2015	SO565	*Campylobacter jejuni* (wild type strain)	Enrichment: Detected in 25 g	Detected in 25 g	Detected in 25 g	Detected in 25 g	Detected in 25 g	Detected in 25 g	Detected in 25 g
			Enumeration: 8 - 97 cfu/g	30 cfu/g	40 cfu/g	50 cfu/g	40 cfu/g	5 cfu/g[a]	3 cfu/g[b]
	SO566	*Campylobacter jejuni* (wild type strain)	Enrichment: Detected in 25g	Detected in 25 g	Detected in 25 g	Detected in 25 g	Detected in 25 g	Detected in 25 g	Detected in 25 g
			Enumeration: 533 - 10900 cfu/g	5700 cfu/g	6400 cfu/g	7500 cfu/g	6400 cfu/g	3400 cfu/g	2100 cfu/g

[a]Result lower than expected range. Investigated according to laboratory quality system and data entry error identified - should have reported 50 cfu/g.
[b] Result lower than expected range. Investigated according to laboratory quality system - possibly outcompeted by high numbers of other organisms.

Table IVb. Results for the six laboratories for the FSA EQA Scheme for *Campylobacter* spp. enumeration

Distribution Reference	Sample Number	*Campylobacter* spp. present?	Mean absolute z-score for all test labs	Laboratory No. (absolute z-score for each trial)					
				1	2	3	4	5	6
PT5	1	Yes	0.80	1.54	0.63	0.10	0.23	0.34	1.51
	2	No	N/A	2.29	-	-	-	-	-
	3	Yes	0.81	1.58	0.60	0.36	0.11	0.39	0.77
	4	Yes	0.71	None provided as reported as greater than result	None provided as reported as greater than result	None provided as reported as greater than result	0.47	0.35	None provided as reported as greater than result
	5	Yes	-	2.10	-	-	-	-	-
	6	Yes	0.75	0.05	0.26	0.53	0.22	0.53	1.52
PT6	1	Yes	0.84	0.02	0.56	0.34	1.13	2.08	1.13
	2	Yes	0.78	1.19	0.77	0.54	1.38	0.04	0.62
	3	Yes	0.81	0.45	0.68	1.27	1.05	1.05	1.27
	4	Yes	0.75	0.32	0.73	0.18	1.92	0.20	0.06
	5	No	-	-	-	-	-	-	-
	6	Yes	0.74	0.09	2.17	0.51	0.02	0.10	0.23
PT7	1	Yes	0.651	0.299	1.995	1.629	0.266	0.964	Low outlier*
	2	Yes	0.355	0.012	0.073	0.332	0.900	0.900	Low outlier*
	3	No	-	-	-	-	-	-	-
	4	Yes	0.506	0.049	0.049	0.555	0.383	0.814	Low outlier*
	5	Yes	0.404	0.054	0.188	1.242	0.034	0.296	Low outlier*
	6	Yes	0.545	0.203	0.203	0.339	0.242	1.688	Low outlier*

*As absolute z-scores are used to determine participants results, for laboratories that record too low or too high a score in the scheme, this results in them being referred to as outliers. In distribution PT7, laboratory 6 had lower results than the other participants resulting in them being given low outlier status when results were returned. This was investigated and dealt with in their Quality System.

APPENDIX V: Descriptive and additional analysis

Enumeration of *Campylobacter* spp. on chickens

The results of the enumeration of *Campylobacter* spp. in the 4,011 chicken skin samples as performed using methods based on EN/ISO 10272-2 are described. Of these, 1,069 (26.7%) were negative for *Campylobacter* spp. The 2,942 positive samples had a median count of 260 cfu per g, with a lower and upper quartile of 60 and 1160 cfu per g, respectively. The fact that the mean of 5,631 cfu per g in the enumerated samples is much greater than the median is an indication of the positive skewness in the distribution of these results. The distribution of the positive samples is described in Table IVa below by the use of estimated centiles of this conditional distribution.

Table Va: Estimated centiles for the 2,942 chickens positive for *Campylobacter* spp.

Centile	estimated cfu per g	95% confidence interval
5	10	10 to 10
10	20	20 to 30
25	60	60 to 70
50	260	240 to 290
75	1,163	1,000 to 1,618
90	5,388	4,500 to 6,181
95	15,000	11,200 to 19,000

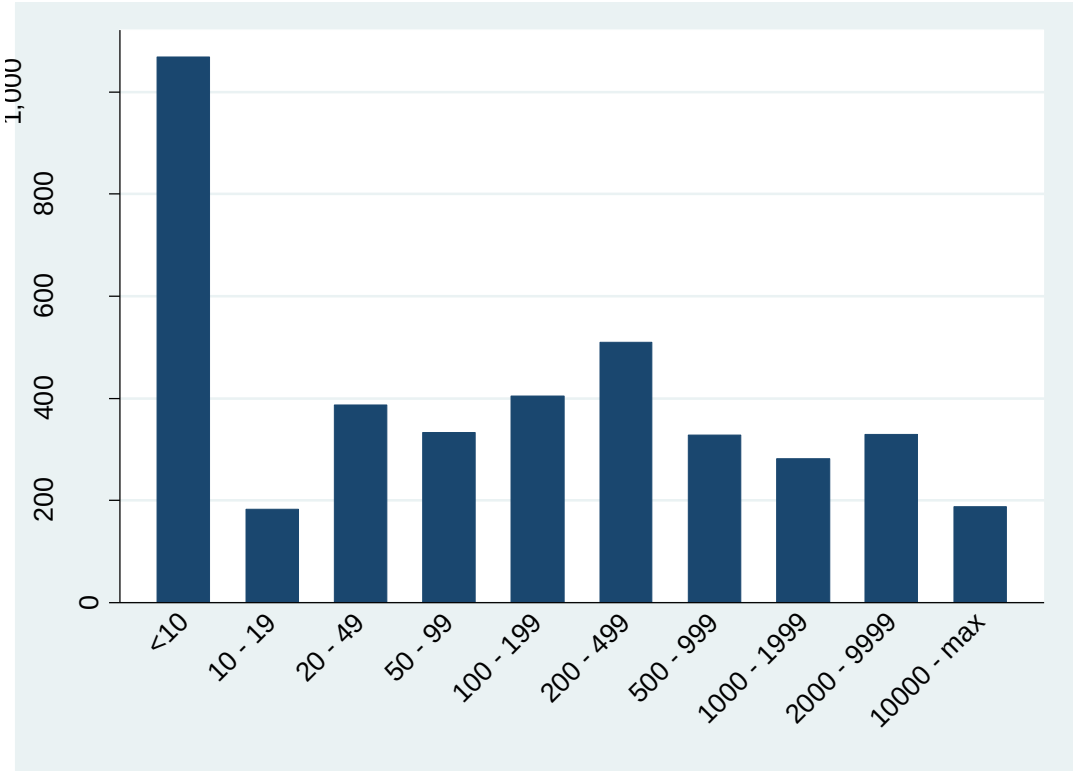

Figure Va: Distribution of cfu of *Campylobacter* spp. per g of chicken skin in samples

The skewness and large proportion of negative results preclude a simple histogram to depict the distributions of the enumeration results. Therefore, a bar chart of categories of enumeration results is shown in Figure IVa to provide a graphical representation of the distribution of *Campylobacter* spp. on chicken skin samples. Some care must be taken in the interpretation of this bar chart due to the uneven category widths.

For the enumeration results obtained by swabbing the packaging of 4,005 chickens, 3,733 (93.2 %) of swabs were negative. Figure IVb shows the distribution of cfu per swab obtained from the outer packaging.

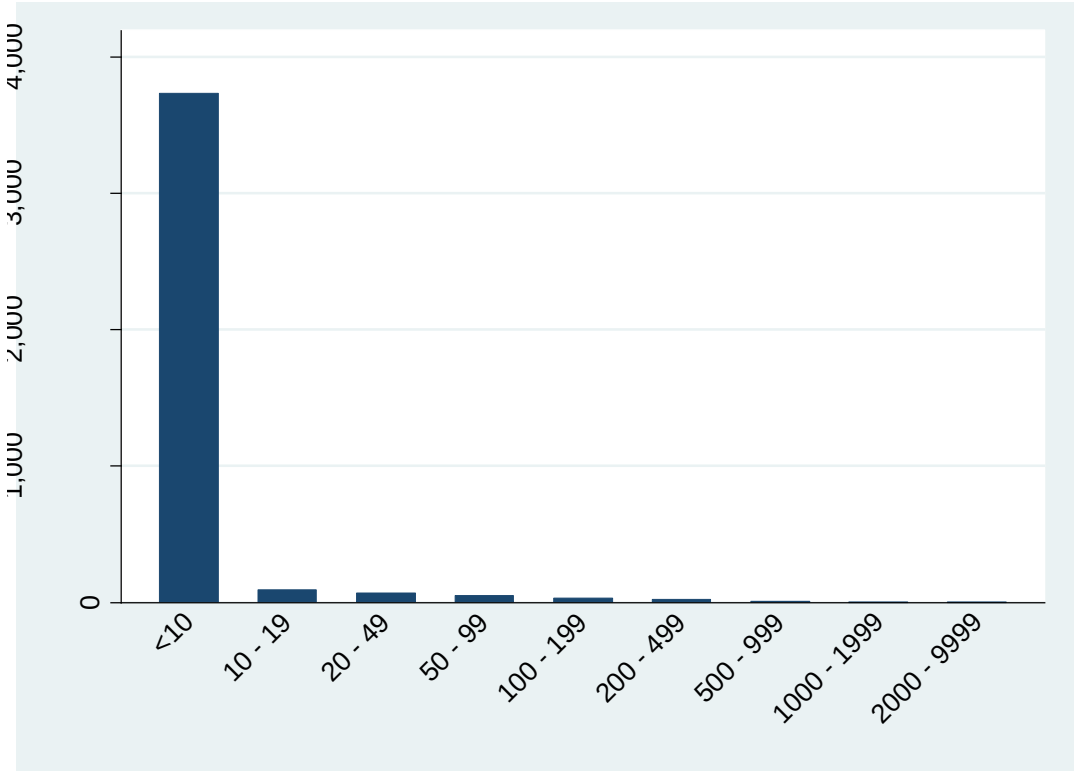

Figure Vb: Distribution of cfu per g of *Campylobacter* spp. on packaging

Direct comparison of the level of contamination in the skin sample with the corresponding outer packaging sample is presented in Table Vb.

Table Vb: Comparison of *Campylobacter* contamination between skin and outer packaging samples from the study chickens.

Campylobacter spp. on outer packaging skin (cfu per swab)	*Campylobacter* spp. contamination on skin (cfu per g skin)				
	<10	10-99	100-1000	>1000	Total
<10	1,053	851	1,172	653	3,729
10-99	10	44	69	85	208
100-1000	1	6	15	36	58
>1000	1	0	0	4	5
Total	1,065	901	1,256	778	4,000

Although the microbiological results show a much reduced level of cfu of *Campylobacter* spp. in the outer packaging compared to the chicken skin samples, there is a significant association, although moderate in magnitude, between the contamination found on the outer packaging and the skin (Spearman's rank correlation 0.19, $p<0.0001$). This could suggest that the source of the contamination on the outer packaging is the chicken within the packaging. However, for a small proportion of the *Campylobacter* contaminated outer packaging samples contamination was absent in the corresponding chicken skin, or as shown in Table 20 the isolated species differed. Thus, there is evidence that for a proportion of the contamination found on the outer packaging the source is unlikely to originate from the chicken inside the pack.

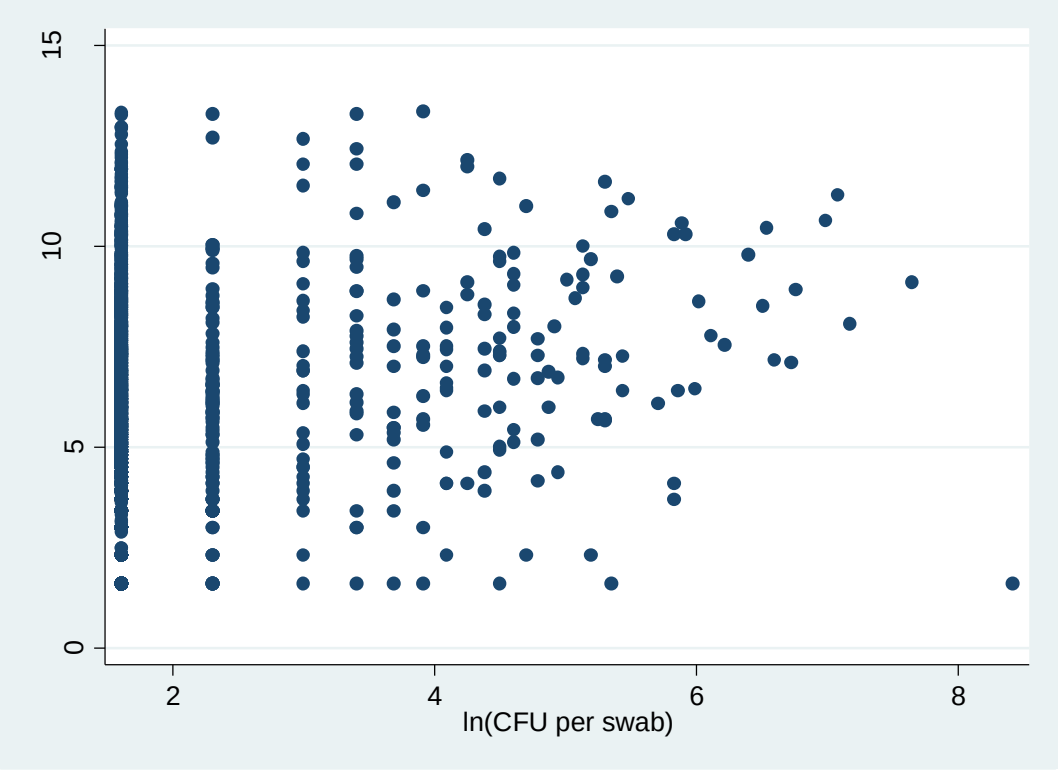

Figure Vc: Scatter plot of the natural logarithm of the enumeration results* from the skin and outer packaging (* skin and swab samples where no *Campylobacter* was detected (<10 cfu) were set to 5 for the purposes of this scatter plot.)

There was a moderate, although statistically significant correlation between the number of cfu found on the skin and the outer packaging of samples, (Pearson correlation coefficient 0.21, p<0.001)

Weight of chickens surveyed

The weight was available for 3,964 of the chickens surveyed. The mean and standard deviation of the weight was 1586 g, and 318 g, respectively. The smallest bird was 350 g, the largest 4,408 g. Table Vc provides the estimated centiles of the weight distribution.

Table Vc: Estimated centiles for the 3,964 chickens with a recorded weight.

Centile	*estimated cfu per g*	*95% confidence interval*
5	1,106	1,100 to 1,120
10	1,200	1,200 to 1,218
25	1,350	1,350 to 1,354
33	1,440	1,420 to 1,450
50	1,550	1,546 to 1,566
66	1,682	1,668 to 1,698
75	1,764	1,750 to 1,788
90	2,000	1,976 to 2,000
95	2,082	2,055 to 2,136

The distribution of weights is to a small extent positively skewed as can be seen in Figure Vd.

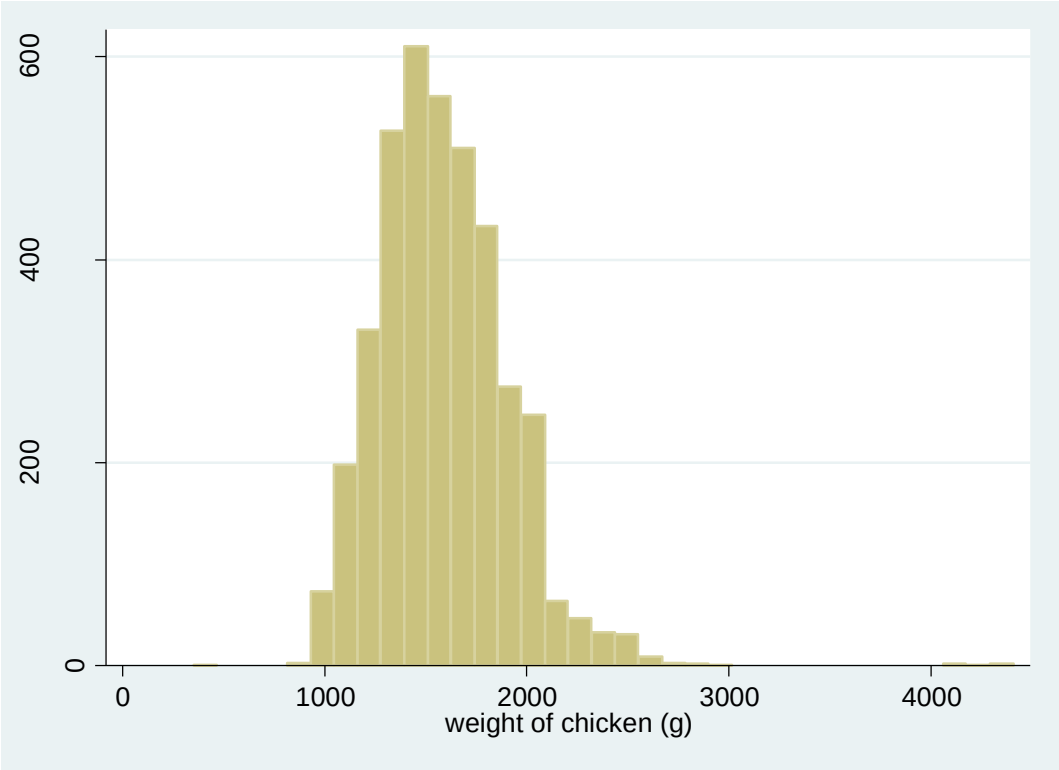

Figure Vd: Distribution of the weight of the surveyed chickens

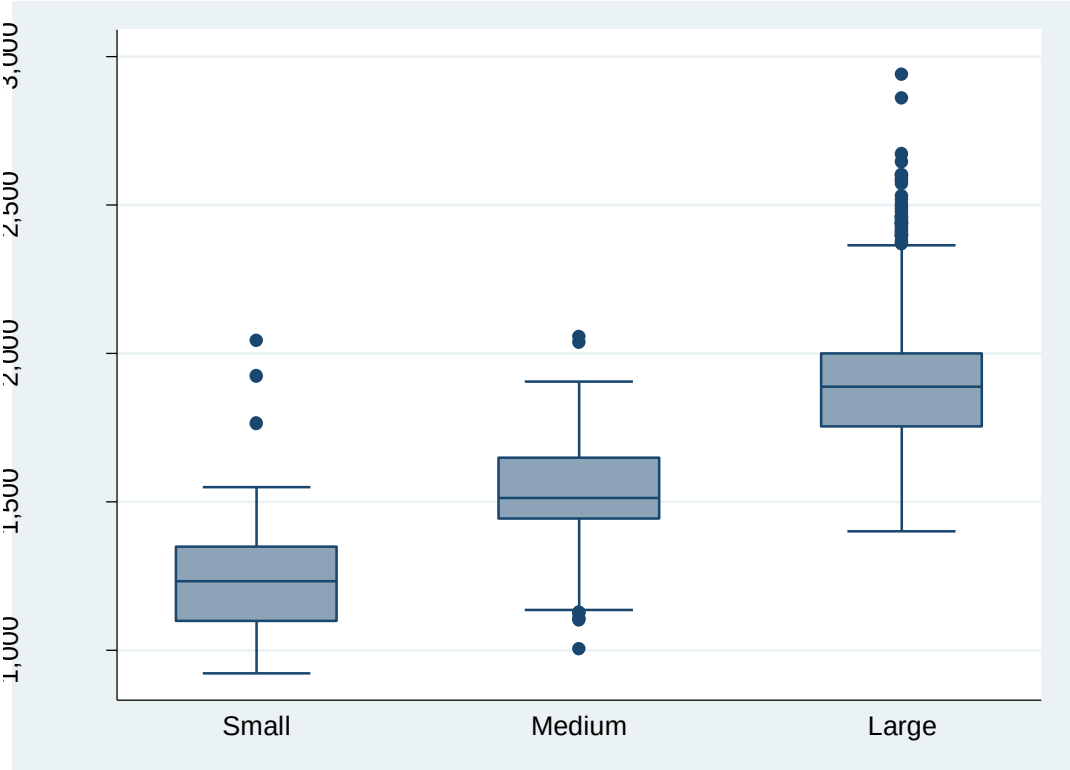

Figure Ve: Box plot of weight of the surveyed chickens (g) by declared size

For 2,148 birds the size, as declared on the packaging, was recorded, with 498 (23.1 %), 839 (39.1 %), and 811 (37.8 %) being labelled as small, medium and large, respectively.

The categories of weights used in the inferential analyses were <1400 g, 1400 to 1750 g, and >1750 g. As can be seen in Figure Ve, there is some overlap in the actual weight of the birds declared to be small, medium, and large, For the birds declared to be small only 7 (1.4 %) weighted more than 1400 g, although 127 (15.2 %) of the birds declared as medium weighed less than 1401 g. For the birds declared to be medium only 16 (1.9 %) weighted more than 1750 g, although 202 (24.9 %) of the birds declared as large weighed less than 1751 g.

Purchase price

A total of 3,977 chickens had their purchase price recorded, these ranging from £2.00 to £21.09, with a mean price of £4.90 (standard deviation £1.51). The distribution of purchase price is shown in Figure Vf.

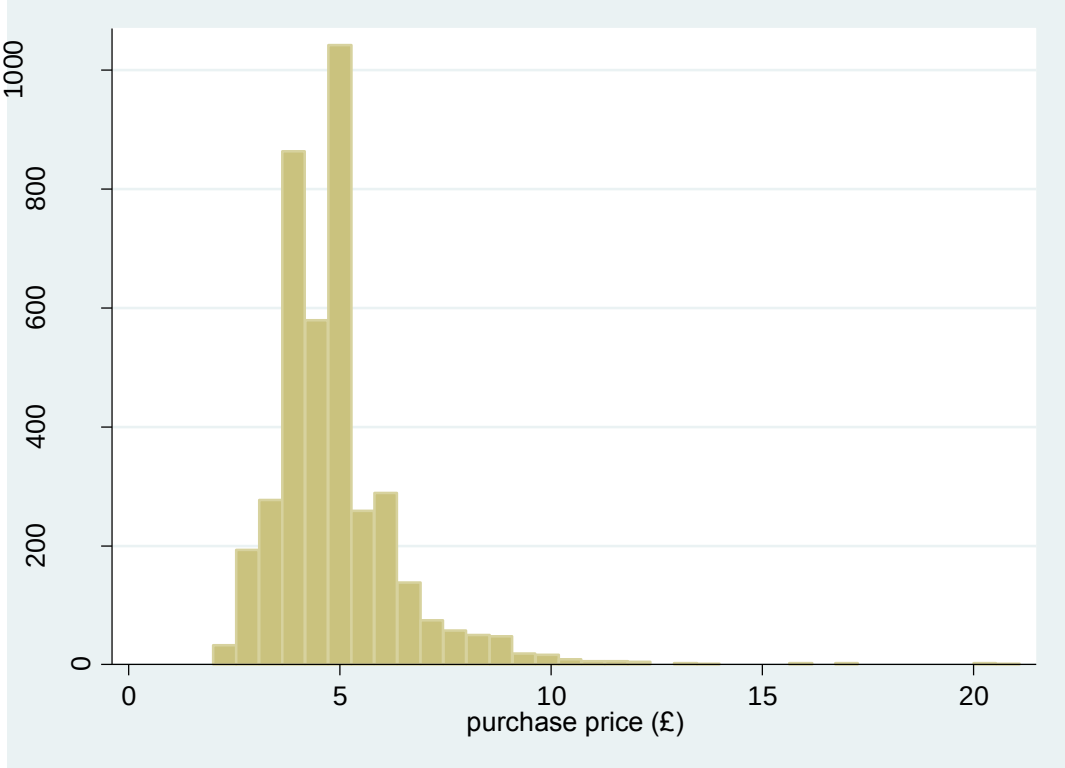

Figure Vf: Distribution of purchase price of the surveyed chickens (£)

As shown in Figure Vg, there is a strong association between the weight of the chicken and its purchase price. In addition there is a strong association between the type of chicken and purchase price. The mean (standard deviation) purchase price for free range, organic, and standard chickens is £7.77 (£2.56), £9.31 (£1.82), and £4.65 (£1.05), respectively.

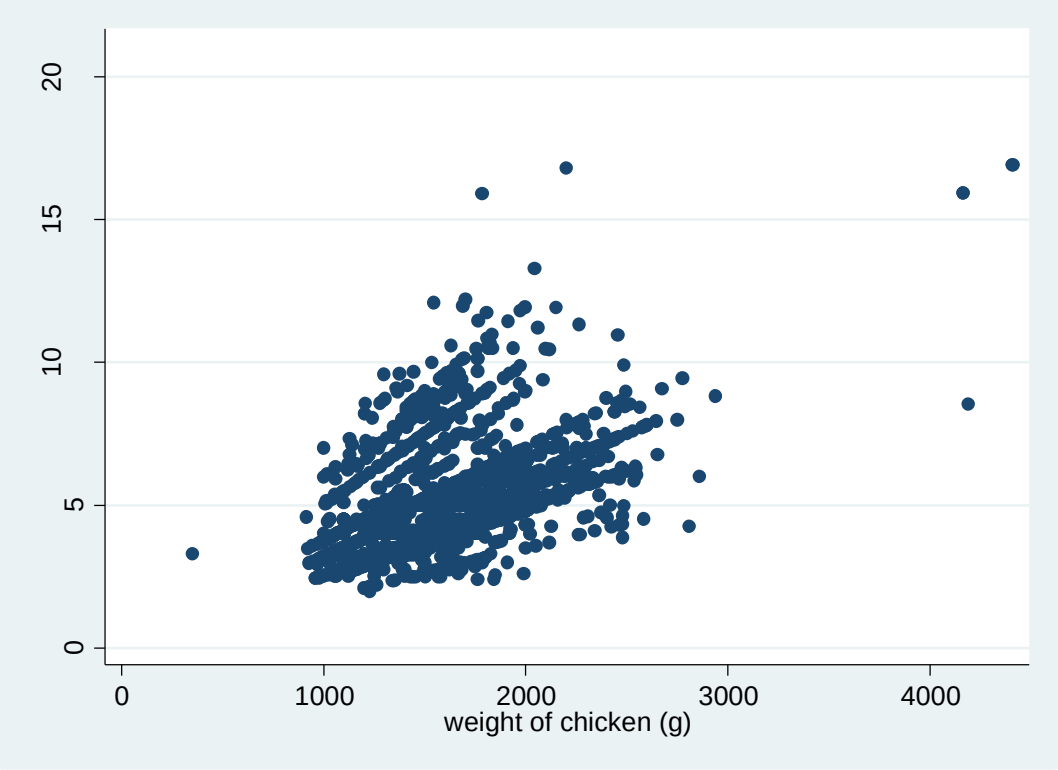

Figure Vg: Scatter plot of purchase price (£) against weight of chickens (g)

Percentage of breast skin used in microbiological method

The EN/ISO 10272-2 requires 25 g of neck skin to be sampled. It is often the situation that there is less than this amount of neck skin in total, and the remainder of the sample for testing is made up of breast skin.

Table Vd: Number (percentage) with breast skin in sample analysed by retailer.

Retailer	percentage breast skin			
	0%	Up to 20%	>20%	Total
ASDA	289 (61.8%)	59 (12.6%)	120 (25.6%)	468
M&S	60 (61.2%)	10 (10.2%)	28 (28.6%)	98
Morrisons	163 (68.5%)	33 (13.9%)	42 (17.7%)	238
Others	266 (67.5%)	45 (11.4%)	83 (21.1%)	394
Sainsbury's	278 (71.8%)	46 (11.9%)	63 (16.3%)	387
Tesco	580 (66.9%)	111 (12.8%)	176 (20.3%)	867
The Co-operative	180 (70.9%)	25 (9.8%)	49 (19.3%)	254
Waitrose	46 (59.7%)	11 (14.3%)	20 (26.0%)	77
Total	1,862 (66.9%)	340 (12.2%)	581 (20.9%)	2,783

For 2,783 chickens the weight of breast skin used in the sample was recorded with confidence (some records were mistakenly recorded as the total number of grams in the sample and no weight could be ascertained for the proportion of neck skin in these samples). Of the 2,783samples 1,862 (66.9 %) used no breast skin, with a contamination rate of 23.5 % (95 % confidence interval 21.5 to 25.4 %) with >1000 cfu per g. A total of 340 (12.2 %) of these samples used up to 5 g (20 %) breast skin and these had a slightly lower, but not significantly different contamination rate of 21.5 % (95 % confidence interval 17.1 to 25.9 %) with >1000 cfu per g. There were 581 (20.9 %) of these samples that contained more than 5 g of breast skin. In these

the contamination rate was significantly lower than in the other two categories being 11.9 % (95 % confidence interval 9.3 to 14.5 %) with >1000 cfu per g.

There were some slight variation in the proportions of samples containing breast skin in the different retailers, which is shown in Table Vd.

Temperature
The temperature of the chicken at collection and the temperature of the air in the transportation box on receipt at the laboratory was recorded precisely for 1,675 and 2,644 chickens, respectively.

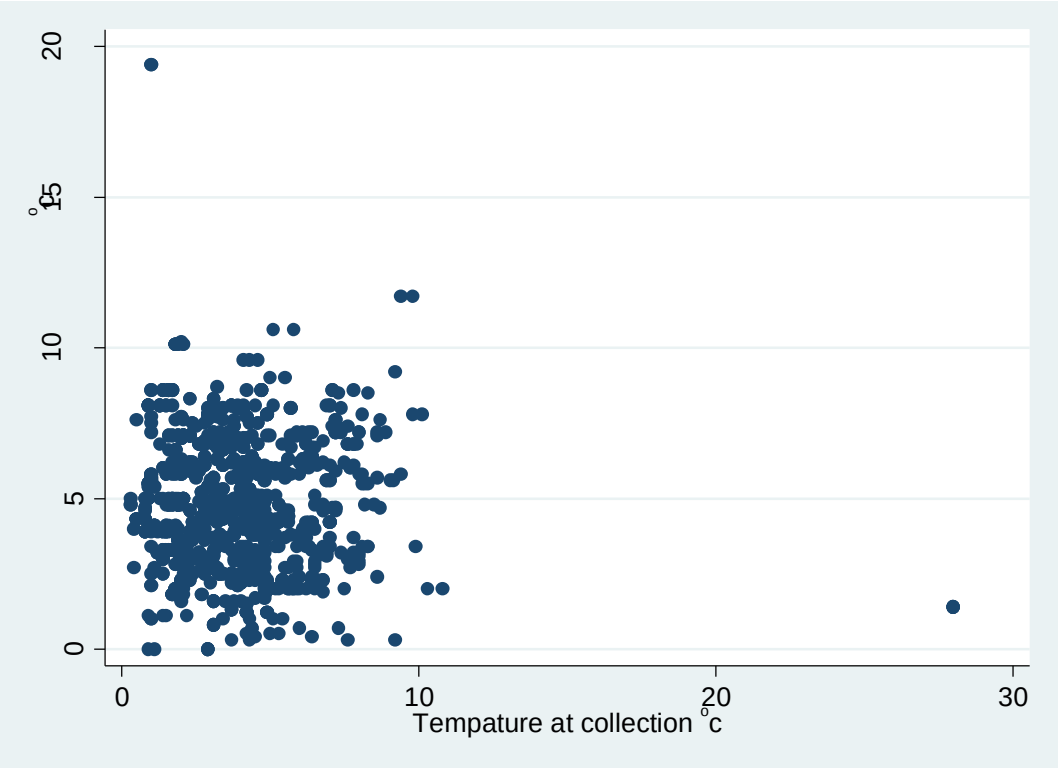

Figure Vh: Scatter plot of temperature on receipt against temperature at collection

There was no significant difference in the proportion of chickens contaminated with >1000 cfu per g between those that had a temperature greater than 8 °C and those with a temperature less than this at collection (p=0.7) or on receipt (p=0.9). At collection 11 (19 %) of the 58 chickens with a temperature greater than 8 °C had > 1000 cfu per g, compared to 276 (17 %) of the1,616 chickens with a temperature less than or equal to 8 °C having >1000 cfu per g skin). On receipt 32 (22.4 %) of the 143 chickens with a temperature greater than 8 °C had >1000 cfu per g, compared to 543 (21. 8 %) of the 2,496 chickens with a temperature less than or equal to 8 °C having > 1000 cfu per g skin.

APPENDIX VI: Logistic regression analysis of presence or absence of *Campylobacter* spp. on chicken skin.

Table VIa. Estimated odds ratios from single variable and multivariable logistic regression models of the presence of *Campylobacter* spp. on chicken skin (contamination levels of 10 or more 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	
Asda	1.09 (0.80-1.49)		1.26 (0.91-1.74)	
M&S	0.57 (0.37-0.90)		0.62 (0.39-0.99)	
Morrisons	0.82 (0.57-1.16)		0.89 (0.62-1.28)	
Others	0.91 (0.67-1.25)		0.97 (0.69-1.36)	
Sainsbury's	0.62 (0.45-0.84)		0.70 (0.50-0.97)	
Tesco	0.54 (0.41-0.70)		0.58 (0.43-0.77)	
Waitrose	0.78 (0.48-1.28)		1.08 (0.64-1.84)	
Chicken type		0.005		0.002
Standard	Reference		Reference	
Free Range	1.41 (1.05-1.89)		1.44 (1.04-1.99)	
Organic	0.43 (0.20-0.90)		0.34 (0.15-0.75)	
Season		<0.001		<0.001
Spring	0.76 (0.63-0.92)		0.79 (0.64-0.96)	
Summer	2.40 (1.94-2.97)		2.51 (2.00-3.13)	
Autumn	1.49 (1.21-1.82)		1.48 (1.20-1.83)	
Winter	Reference		Reference	
Remaining shelf-life		0.01		<0.001
Per additional day	1.05 (1.01-1.10)		1.10 (1.05-1.15)	
Weight		<0.001		<0.001
Small <1400 g	Reference		Reference	
Medium 1400-1750 g	1.83 (1.56-2.15)		1.72 (1.44-2.05)	
Large >1750 g	2.78 (2.28-3.40)		2.70 (2.19-3.33)	

The results obtained for the association between the presence of *Campylobacter* spp. on the chicken skins and various factors are presented in Table VIa. These are generally similar to those presented in Table 8 for high level contamination. However, the association with retailer is less clear when assessing presence of *Campylobacter* spp. although ASDA remains the retailer with the highest rate, and Tesco the lowest. The association with season is more clear-cut, with a sharper increase in the presence of *Campylobacter* spp. seen in the summer. Using the presence of campylobacters as a measure of contamination a higher proportion of free-range compared to standard or organic chickens were positive for campylobacters.