

FSA Project FS102121

Year 3 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, Nicolae Corcionivoschi and Nicola C Elviss

© Crown Copyright 2018

This report has been produced by Public Health England (PHE) under a Memorandum of Understanding placed by the Food Standards Agency (FSA). The views expressed herein are not necessarily those of the Agency. PHE warrants that all reasonable skill and care has been used in preparing this report. Notwithstanding this warranty, PHE shall not be under any liability for loss of profit, business, revenues or any special indirect or consequential damage of any nature whatsoever or loss of anticipated saving or for any increased costs sustained by the client or his or her servants or agents arising in any way whether directly or indirectly as a result of reliance on this report or of any error or defect in this report.

Contents

Ackno	owledgements4
Abbre	viations5
Execu	itive summary6
1.0	Background8
2.0	Methods
2.1	Sampling method12
2.2	Microbiological methods12
2.3	Quality Assurance
2.4	Statistical Analysis
3.0 Re	esults14
Figure	e 1. Geographical distribution of samples collected for the survey14
3.1	Number of <i>Campylobacter</i> in chicken skin samples from whole fresh UK produced chicken. 15
3.1.1	Campylobacter spp. in chicken skin samples in relation to retailer15
Table	1. Number of <i>Campylobacter</i> spp. in retail chicken in relation to retailer15
3.1.2 rearin	Number of <i>Campylobacter</i> spp. in chicken skin samples in relation to chicken g regime16
Table	2. Number of Campylobacter spp. in chicken in relation to bird rearing regime 16
3.1.3 proce	Number of <i>Campylobacter</i> spp. in chicken skin samples in relation to chicken ssor approval number16
Table	3. Number of <i>Campylobacter</i> spp. in retail chicken in relation to processor 17
3.1.4	Number of <i>Campylobacter</i> spp. in chicken skin samples in relation to season. 18
Table	4. Number of Campylobacter spp. in retail chicken in relation to sample time18
3.1.5 weigh	Number of <i>Campylobacter</i> spp. in chicken skin samples in relation to chicken t 18
3.1.6 shelf-	Number of <i>Campylobacter</i> spp. in chicken skin samples in relation to days of life remaining18
Table 	5. Number of <i>Campylobacter</i> spp. in retail chicken in relation to chicken weight19
	6. Number of <i>Campylobacter</i> spp. in retail chicken in relation to days of ning shelf-life19
3.1.7	Other factors19
3.2	Logistic regression
regres	7. Estimated odds ratios from single variable and multivariable logistic ssion models of <i>Campylobacter</i> spp. contamination levels >1000 cfu per g

3.3 reta	теления и предоставления и предоставлени	ı at
Table	9. Campylobacter spp. isolates from retail chicken skin samples	22
	10. <i>Campylobacter jejuni</i> and <i>C. coli</i> isolates from retail chicken skin sample on to season	
	11. <i>Campylobacter jejuni</i> and <i>C. coli</i> isolates from retail chicken skin sample on bird rearing regime	
	12. <i>Campylobacter jejuni</i> and <i>C. coli</i> isolates from retail chicken in relation to	
4.0	Discussion	24
4.1	Survey results	24
4.2	Human campylobacter infections in the UK	25
	13. Number and rate* of reported campylobacter infections in the United domain and by country per 100,000 population, 2006-2016	26
4.3	Conclusions	26
5.0	References	27

Acknowledgements

The authors would like to say thank you to the following people:

All staff who were involved in the successful delivery of this project from PHE and AFBI through the sampling and testing of chickens

Mike Hutchison and colleagues in Hutchison Scientific

Abrar Jaffer, Lorna Rowswell and colleagues at the Food Standards Agency

Colleagues in GBRU at PHE Colindale for speciation data

Colleagues in PHE Statistics Unit

Lesley Larkin and Richard Elson for support with epidemiological data

Abbreviations

°C Degrees Celsius

GBRU Gastrointestinal Bacteria Reference Unit

CI Colony forming units
CI Confidence Interval

EQA External Quality Assurance
FSA Food Standards Agency

g Gramh Hour(s)

PHE Public Health England

IQA Internal Quality Assurance

ISO International Standard Organisation

I Litre

Laboratory Information Management System

mCCDA modified Charcoal Cefoperazone Deoxycholate Agar

mg Milligram Millilitre

MRD Maximum Recovery Diluent

n NumberOR 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 neck skin) to below 10 % at the end of the slaughter process, initially by the end of 2015 but this was rolled over to 2016.

A UK-wide survey was undertaken to determine the levels of *Campylobacter* spp. on whole fresh retail chickens. The first survey year of data was collected by FSA Project FS241044 (2014/15) and this report represents results from sampling and testing chickens in the third survey year under FSA Project FS102121 (2016/2017).

A total of 4268 samples of whole, UK-produced, fresh chicken were tested between August 2016 to July 2017 during this third survey year. The samples were distributed throughout the UK (in proportion to the population size of each country) and testing was performed by four laboratory sites; three Public Health England (PHE) laboratories 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.

Campylobacter enumeration testing on chicken samples was performed using the EN/TS/ISO 10272-2 standard enumeration method applied with a detection limit of 10 colony forming units (cfu) per gram of neck skin.

The proportion of *Campylobacter* spp. in fresh, whole chicken at retail in the UK in the survey period from August 2016 to July 2017 was 54 %. Also, in this time period, 6 % of samples had > 1000 cfu per g chicken skin (highly contaminated chicken category).

There were significant differences in the proportion of highly contaminated chickens (ranging from 1 to 18 %) between the retailers that could not be explained by differences in remaining shelf-life, chicken weight, sampling period or the type of rearing used. Comparison of 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 to 19 %, and it was noted that some retailers were predominantly supplied by specific approved slaughter premises.

The larger chickens, those with >1750 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 comparisons made.

For the majority of chicken skin samples (87.7 %) from which isolates were submitted for speciation (n = 2167), *C. jejuni* alone was identified. *Campylobacter coli* alone was identified in 10.2 % of samples. Both species were found in 1.9 % of

samples. *Campylobacter coli* was more frequently isolated in the summer months and from birds with access to range.

The average proportion of fresh, whole chicken at retail sale in the UK that are contaminated with a high level of campylobacters has decreased considerably but chickens from the group of shops comprising smaller retailers, independents and butchers remain highly contaminated, suggesting more action is needed to achieve better control of *Campylobacter* spp. in those chickens. Data from this year and the previous survey years have demonstrated an overall substantial 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 can demonstrate a sustained decline.

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, data published previous to the FSA survey lacked 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 gram (g), from a 2008 baseline of 27 % to less than 10 % by December 2015; this target was rolled over to 2016. 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. Enzyme Linked Immunosorbent Assay (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).

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). Industry (with support from 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 Joint Working Group on Campylobacter (JWG). The JWG then developed into 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 was found to be 73.3 %. A significant proportion (19.4 %) of samples had > 1000 cfu per g chicken skin, and this ranged between all retailers from 12.9 to 29.9 %. Overall. campylobacters were detected from the outer-packaging swab in 6.8 % of samples. The Campylobacter spp. contamination found on the outer packaging was at low levels, but 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 (reflecting market share) 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, therefore Campylobacter spp. in chicken continued to be important in terms of foodborne disease risk. These findings led to the FSA continuing the monitoring programme over three further years (under project FS102121), aiming 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. The project also was to continue to identify *Campylobacter* spp. present and determine susceptibility of isolates to a defined range of antimicrobial agents (published as a separate report).

In the second survey year, the prevalence of *Campylobacter* spp. in the fresh chicken at retail in the UK had declined to 61.3 % and the proportion of samples with > 1000 cfu per g chicken skin to 11.4 %. 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. 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 were highly contaminated with *Campylobacter* spp. during the first summer months compared to the subsequent months. The larger chickens (ie. those weighing > 1750 g) 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.

Data from the first two survey years has therefore demonstrated a significant decline in the level of highly contaminated fresh whole UK retail chicken.

The purpose of examining numbers of campylobacters in fresh chicken on retail sale in the UK for a third survey year was to determine whether a sustained decline in contamination remained evident.

2.0 Methods

Sampling and testing procedures for the survey and the method evaluation work was agreed with the FSA. The survey protocol used for the time-period from August 2016 to July 2017 is briefly described (FSA 2016 and enclosed as Appendix I).

2.1 Sampling method

Sampling was spread across the UK and designed to reflect population sizes. A similar number of samples were obtained from each retailer, an approach also used during the second survey year (PHE 2017). The numbers of free-range and organic chickens sampled within these were based on market share data from Kantar (FSA 2016). Samples for the survey were collected by trained individuals, who purchased samples from retail outlets and transported them to the appropriate testing laboratory according to the survey 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

Four laboratories undertook the testing during the survey period; three 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 2016) using modified Charcoal Cefoperazone Deoxycholate Agar as the primary plating medium. All participating laboratories used the same method of achieving a microaerophilic atmosphere (Campygen sachets, Thermofisher Ltd.).

Neck-skin samples were prepared as described before (also see Appendix I) using a 1:9 dilution of chicken neck-skin and buffered peptone water. Sample weights were between 2 to 10 g pure neck-skin. In contrast to the previous survey year no breast-skin was added as a small but significant effect on the level of campylobacters was detected.

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. The pilot provided the basis on which the current survey of whole UK-produced fresh retail chicken was developed. The amended weight of neck skin was validated at the end of survey year 2, from 25 g of neck skin to 5-10 g. This was carried out due to changes in chicken production resulting in increased amounts of neck skin being removed.

All laboratories participate in recognised External Quality Assurance schemes (e.g. https://www.gov.uk/government/collections/external-quality-assessment-eqa-and-proficiency-testing-pt-for-food-water-and-environmental-microbiology) 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. Fisher's exact test was used for individual comparisons when samples were small. 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 (termed quarters).

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 August 2016 and July 2017 (Figure 1).

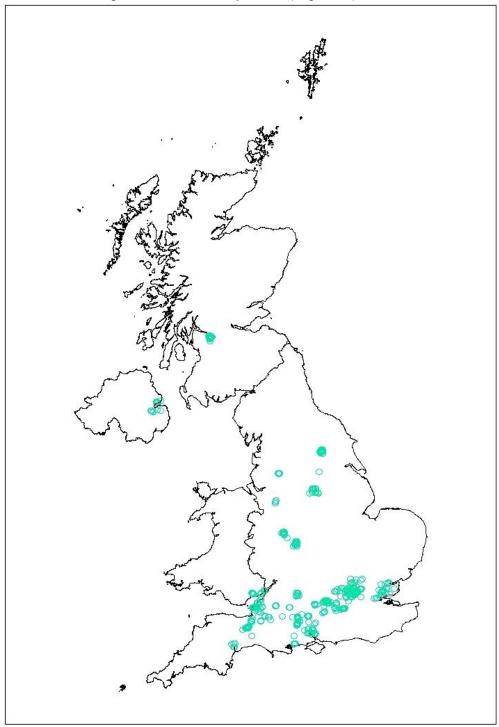


Figure 1. Geographical distribution of samples collected for the survey

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.

3.1 Number of *Campylobacter* in chicken skin samples from whole fresh UK produced chicken.

Based on all chickens examined during the survey period from August 2016 to July 2017, *Campylobacter* spp. were detected in the majority (54 %) of chicken skin samples and 6 % (95 % CI = 5 to 7 %) of the samples had counts above 1000 cfu per g chicken skin. The highest single count detected was 565,000 cfu of *Campylobacter* per g chicken skin.

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 2 to 18 % across the retailer groups (Table 1). For the nine larger retailers (i.e. Aldi, Asda, Co-op, Lidl, M&S, Morrisons, Sainsbury's, Tesco and Waitrose) only between 2 and 8 % of chicken had *Campylobacter* spp. levels of > 1000 cfu per g. Shops such as Butchers and Other (smaller) retailers tended to have higher proportions of chicken with > 1000 cfu per g of *Campylobacter* spp. (Table 1).

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

		cfu of C	Campylo	ampylobacter spp. per g chicken skin sample							
Retailer	<10		10-99		100-1000		> 1000				
(n*)	n	%	n	%	N	%	n	%			
		(95 % CI)		(95 % CI)		(95 % CI)		(95 % CI)			
Aldi (453)	204	45 (40 – 50)	127	28 (24-32)	96	21 (18-25)	26	6 (4-8)			
Asda (413)	175	42 (38-47)	113	27 (23-32)	94	23 (19-27)	31	8 (5-10)			
Co-op (423)	202	48 (43-53)	119	28 (24-33)	84	20 (16-24)	18	4 (3-7)			
Lidl (425)	184	43 (39-48)	119	28 (24-33)	93	22 (18-26)	29	7 (5-10)			
M&S (441)	170	39 (34-43)	125	28 (24-33)	119	27 (23-31)	27	6 (4-9)			
Morrisons (411)	202	49 (44-54)	128	31 (27-36)	69	17 (13-21)	12	3 (2-5)			
Sainsbury's (425)	201	47 (42-52)	120	28 (24-33)	81	19 (15-23)	23	5 (3-8)			
Tesco (435)	234	54 (49-59)	124	29 (24-33)	60	14 (11-17)	17	4 (2-6)			
Waitrose (464)	282	61 (56-65)	132	28 (24-33)	40	9 (6-12)	10	2 (1-4)			
Others# (182)	45	25 (19-32)	32	18 (12-24)	72	40 (32-47)	33	18 (13-25)			
Butcher (196)	67	34 (28-41)	43	22 (16-28)	60	31 (24-38)	26	13 (9-19)			
Total (4268)	1966	46 (45-48)	1182	28 (26-29)	868	20 (19-22)	252	6 (5-7)			

^{*}n = Number of samples

^{*}Others included supermarkets with lower market shares and independents e.g. Iceland, convenience stores.

The proportion of chickens with *Campylobacter* spp. levels at >1000 cfu per g differed significantly (as evidenced by Chi-square test and non-overlapping 95 % Cl) between some of the retailers (Table 1). Possible confounding of these results was examined using logistic regression (see section 3.2).

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

The rearing regime for chickens examined was recorded, and Table 2 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. 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 2. Number of *Campylobacter* spp. in chicken in relation to bird rearing regime

Dearing		cfu of C	ampylo	obacter spp.	per g c	hicken skin s	ample)
Rearing	<10		10-99		100-1000		>1000	
regime (n*)	n	% (95 % CI)	n	% (95 % CI)	n	% (95 % CI)	n	% (95 % CI)
Standard (3812)	1765	46 (45-48)	1050	28 (26-29)	772	20 (19-22)	225	6 (5-7)
Free Range (417)	185	44 (40-49)	117	28 (24-33)	89	21 (18-26)	26	6 (4-9)
Organic (39)	16	41 (26-58)	15	38 (23-55)	7	18 (8-34)	1	3 (0-13)

^{*}n = Number of samples

3.1.3 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; Table 3). The percentage of chickens with >1000 cfu per g ranged from 1 % for approval number 9502 to 19 % for approval number 5007. Approval numbers 3011, 4014 and 9502 produced significantly fewer highly contaminated chickens compared to the average for all samples. Approval numbers 5007, 5450 and the group of other smaller production premises produced more highly contaminated chickens compared to the average of all samples tested.

Table 3. Number of *Campylobacter* spp. in retail chicken in relation to processor.

_		cfu of C	Campyle	obacter spp.	per g d	chicken skin s	sample	9
Processor Approval		<10	10-99		•	100-1000	>1000	
number (n*)	n	% (95 % CI)	n	% (95 % CI)	n	% (95 % CI)	n	% (95 % CI)
1100 (272)	137	50 (44-56)	57	21 (16-26)	55	20 (16-25)	23	8 (5-12)
2037 (464)	216	47 (42-51)	150	32 (28-37)	74	16 (13-20)	24	5 (3-8)
3005 (322)	133	41 (36-47)	101	31 (26-37)	70	22 (17-27)	18	6 (3-9)
3007 (315)	132	42 (36-48)	79	25 (20-30)	80	25 (21-31)	24	8 (5-11)
3011 (231)	157	68 (62-74)	55	24 (18-30)	15	6 (4-10)	4	2 (0-4)
4014 (369)	209	57 (51-62)	112	30 (26-35)	42	11 (8-15)	6	2 (1-4)
5007 (113)	20	18 (11-26)	20	18 (11-26)	51	45 (36-55)	22	19 (13-28)
5011 (735)	274	37 (34-41)	220	30 (27-33)	200	27 (24-31)	41	6 (4-7)
5450 (80)	12	15 (8-25)	23	29 (19-40)	32	40 (29-52)	13	16 (9-26)
5464 (70)	15	21 (13-33)	26	37 (26-50)	26	37 (26-50)	3	4 (1-12)
8005 (422)	205	49 (44-53)	112	27 (22-31)	86	20 (17-25)	19	5 (3-7)
9502 (560)	354	63 (59-67)	157	28 (24-32)	43	8 (6-10)	6	1 (0-2)
Other code# (302)	97	32 (27-38)	66	22 (17-27)	91	30 (25-36)	48	16 (12-21)
Not Available [§] (13)	5	38 (14-68)	4	31 (9-61)	3	23 (5-54)	1	8 (0-36)
Total (4268)	1966	46 (45-48)	1182	28 (26-29)	868	20 (19-22)	252	6 (5-7)

^{*}n = Number of samples

 $^{^{\#}}$ Samples listed within the 'Other code' category had < 50 chickens from the processor sampled within the study. A <u>list of approved premises codes</u> can be found on the FSA website

[§]Shop was unable to provide processor Approval number

3.1.4 Number of Campylobacter spp. in chicken skin samples in relation to season.

The proportion of samples with > 1000 cfu/g of Campylobacter spp. was not significantly different between the different sampling quarters (Table 4).

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

Quarter	cfu of <i>Campylobacter</i> spp. per g chicken skin sample								
(n*)		<10		10-99		100-1000		>1000	
	n	% (95 % CI)	n	% (95 % CI)	N	% (95 % CI)	N	% (95 % CI)	
1 Aug/Sep/Oct 2016 (1038)	425	41 (38-44)	313	30 (27-33)	236	23 (20-25)	64	6 (5-8)	
2 Nov/Dec 2016 & Jan 2017 (1076)	526	49 (46-52)	266	25 (22-27)	209	19 (17-22)	75	7 (6-9)	
3 Feb/Mar/Apr 2017 (950)	488	51 (48-55)	223	23 (21-26)	190	20 (18-23)	49	5 (4-7)	
4 May/Jun/Jul 2017 (1204)	527	44 (41-47)	380	32 (29-34)	233	19 (17-22)	64	5 (4-7)	

^{*}n = Number of samples

3.1.5 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 described as 'small' or 'medium' or 'large' (Table 5). 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, was associated with the level of *Campylobacter* spp. present. Using these categories large birds had a statistically significantly higher number of samples with >1000 cfu of *Campylobacter* spp. per g (Table 5).

3.1.6 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 6). At testing, the most frequent number of days of shelf-life remaining was 4-5 days. There was a trend suggesting that a high-level contamination was more likely in samples with the longest shelf-life remaining, i.e. there was an association with those birds that were closer to their production date. This was supported by the logistic regression analysis results (section 3.2).

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

		cfu of Campylobacter spp. per g chicken skin sample									
Chicken weight		<10		10-99		100-1000		>1000			
(n*)	n	% (95 % CI)	n	% (95 % CI)	n	% (95 % CI)	n	% (95 % CI)			
Small <1400 g (1501)	852	57 (54-59)	347	23 (21-25)	236	16 (14-18)	66	4 (3-6)			
Medium 1400- 1750 g (1872)	803	43 (41-45)	578	31 (29-33)	390	21 (19-23)	101	5 (4-7)			
Large >1750 g (884)	306	35 (31-38)	257	29 (26-32)	238	27 (24-30)	83	9 (8-12)			
Not stated (11)	5	45 (17-77)	0	0 (0-28)	4	36 (11-69)	2	18 (2-52)			

^{*}n = Number of samples; no weight data was available for 11 chickens.

Table 6. Number of *Campylobacter* spp. in retail chicken in relation to days of remaining shelf-life.

Pomoining		cfu of C	ampyl	obacter spp. p	er g cl	nicken skin s	sampl	е
Remaining shelf-life in		<10		10-99	10	00-1000		>1000
days (n*)	n	% (95 % CI)	n	% (95 % CI)	n	% (95 % CI)	n	% (95 % CI)
0-1 (121)	59	49 (40-58)	32	26 (19-35)	23	19 (12-27)	7	6 (2-12)
2-3 (1066)	569	53 (50-56)	268	25 (23-28)	173	16 (14-19)	56	5 (4-7)
4-5 (1952)	889	46 (43-48)	548	28 (26-30)	403	21 (19-23)	112	6 (5-7)
6-7 (1011)	413	41 (38-44)	296	29 (26-32)	235	23 (21-26)	67	7 (5-8)
8-9 (109)	34	31 (23-41)	33	30 (22-40)	32	29 (21-39)	10	9 (4-16)
Not available (9)	2	22 (3-60)	5	56 (21-86)	2	22 (3-60)	0	0 (0-34)

^{*}n = Number of samples

3.1.7 Other factors

Whilst the protocol stipulated to test a 10 g neck-skin sample not all chickens had sufficient neck-skin available for 10 g to be tested and were then tested with sample weights from 2 to < 10 g. The average grams of neck-skin in samples did not differ significantly between retailers, with Aldi having the highest (8.6 g) average amount in samples. While 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, data from the previous survey year (PHE 2016) 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. This issue was also addressed further as part of the logistic regression analysis described below (3.2) Some retailers consistently sold chickens packed using a modified atmosphere packaging (MAP) whilst the large majority of chickens obtained from butchers and

other non-major retailers were not MAP packed. MAP packing was therefore highly correlated with retailer type. For a proportion of the 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).

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 7). In the approach used for the regression the same variables were retained as used in the previous years to make the results comparable over the years. The variables of interest were also included based on the extent to which they lent themselves to statistical analysis.

This indicated that the variation in the percentage of chickens with the highest level of *Campylobacter* spp. contamination (over 1000 cfu/g) 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 retail outlets including independents and butchers, was 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.

Although a small but significant effect of the amount of neck-skin weight in the sample was found (OR =- 1.14; p< 0.01; logistic regression taking neck-skin weight into account) this did not confound the effect of other factors including retailer or approval number (results not shown).. Both present and past data have indicated that the total weight of the neck-skin sample does not confound other factors (i.e. other factors remain independently significant). However we cannot rule out that total sample weight (which is likely to be associated with the extent to which a processor may remove neck-skin) could have impacted the effect size of other factors, hence caution has to be applied when comparing results.

Table 7. 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 a	nalysis
Variable	OR (95 % CI)	p-value	OR (95 % CI)	p-value
Retailer		<0.0001		<0.0001
Со-ор	Reference		Reference	
Aldi	1.37 (0.74-2.54)		1.14 (0.60-2.16)	
Asda	1.83 (1.01-3.32)		1.77 (0.96-3.25)	
Lidl	1.65 (0.90-3.02)		1.42 (0.76-2.63)	
M&S	1.47 (0.80-2.71)		1.69 (0.90-3.15)	
Morrisons	0.68 (0.32-1.42)		0.55 (0.26-1.17)	
Sainsbury's	1.29 (0.68-2.42)		1.34 (0.70-2.58)	
Tesco	0.92 (0.47-1.80)		0.91 (0.46-1.81)	
Waitrose	0.50 (0.23-1.09)		0.68 (0.31-1.50)	
Other	4.12 (2.41-7.20)		5.19 (2.92-9.20)	
Chicken type		0.665		0.624
Standard	Reference		Reference	
Free Range	1.06 (0.70-1.61)		0.84 (0.54-1.31)	
Organic	0.42 (0.06-3.07)		0.52 (0.07-3.93)	
Quarter ^a		0.260		0.141
Quarter 2	 Reference		Reference	
Quarter 1	0.89 (0.62-1.24)		0.83 (0.59-1.19)	
Quarter 3	0.73 (0.50-1.05)		0.67 (0.46-0.98)	
Quarter 4	0.75 (0.53-1.06)		0.72 (0.50-1.02)	
Remaining shelf-life		0.025		<.0001
Per additional day	 1.10 (1.01-1.19)		1.21 (1.10-1.32)	
Weight		<0.0001		0.0002
Small <1400 g	 Reference		Reference	
Medium 1400-1750 g	1.24 (0.90-1.70)		1.36 (0.98-1.90)	
Large >1750 g	2.25 (1.61-3.15)		2.07 (1.46-2.94)	

^aFor the purposes of this report, Q1 was defined as August-October 2016; Q2 as November 2016-January 2017; Q3 as February-April 2017 and Q4 as May-July 2017.

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

Isolates from a total of 2167 chicken neck skin samples were subjected to *C. jejuni/C. coli* speciation testing. *C. jejuni* alone was found in 87.7 %, *C. coli* alone in 10.2 %, both species in 1.9 % of samples (Table 9). *Campylobacter* spp. (likely to be campylobacters other than *C. coli* or *C. jejuni*) were detected in only 3 samples. No speciation test was available for 135 samples (5.9 % of the total number of *Campylobacter*-positive samples) due to loss of isolate viability.

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

Species detected	No. of samples	% of samples
C. jejuni (only)	1900	87.7
C. coli (only)	222	10.2
C. jejuni and C. coli	42	1.9
Campylobacter spp.	3	0.1

C. coli alone was significantly more frequently isolated during the summer months (13.9 %), compared to the rest of the year (8.8 %) (p < 0.001; Fisher's exact test) (Table 10). Conversely, the proportion of samples from which *C. jejuni* was isolated was lower in the summer months (83.8 %) compared to all other months in the survey period (89.4 %).

Table 10. Campylobacter jejuni and C. coli isolates from retail chicken skin samples in relation to season

	% of samples (number of samples)							
	Se	Season						
Species detected	Summer (August 2016 and June & July 2017) (n = 619)	Autumn, Winter and Spring (September-December 2016 & January-May 2017) (n = 1545)						
<i>C. jejuni</i> only	83.8 (519)	89.4 (1381)						
C. coli only	13.9 (86)	8.8 (136)						
C. jejuni and C. coli	2.2 (14) 1.8 (28)							

The proportion of samples $C.\ coli$ was isolated from where chickens were reared as free-range or organic was significantly higher compared to samples from chickens reared without access to range (termed standard rearing; p < 0.001 and < 0.005 for free-range or organic, respectively; Fisher's exact). However, further data would be required to ascertain this observation for the comparison of the organic reared birds in particular, as only a small number of samples from these birds were tested.

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

	Chicken rearing method % of samples with Campylobacter species (no. of samples)							
Species detected	Standard rearing (no access to range) (n = 1931)	Free range (n = 211)	Organic (n = 22)					
C. jejuni only	90.8 (1753)	63.5 (134)	59.1 (13)					
C. coli only	7.7 (149)	31.3 (66)	31.8 (7)					
C. jejuni and C. coli	1.5 (29)	5.2 (11)	9.1 (2)					

There was evidence of differences in the proportion of *C. coli/C. jejuni* isolates found between different approval numbers. In particular, a higher proportion of *C. coli* appeared to be isolated among certain approval numbers with the highest proportion seen for an approval number known to predominantly produce free-range chickens (Table 12).

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

Processor	<i>C. jejuni</i> only		C. cc	oli only	C. jejuni & C. coli	
Approval Number	%	No. of samples	%	No. of samples	%	No. of samples
1100	96.3	130	3.7	5	0.0	0
2037	95.5	214	4.0	9	0.4	1
3005	96.2	178	2.7	5	1.1	2
3007	96.5	166	1.2	2	2.3	4
3011	88.7	63	9.9	7	1.4	1
4014	90.4	141	7.1	11	2.6	4
5007	77.5	69	19.1	17	3.4	3
5011	89.7	383	8.2	35	2.1	9
5464	84.6	44	15.4	8	0.0	0
5450	61.5	40	36.9	24	1.5	1
8005	88.5	177	10.0	20	1.5	3
9502	79.7	153	16.7	32	3.6	7
Other code#	72.9	137	23.4	44	3.7	7
Not Available§	62.5	5	37.5	3	0.0	0

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

^{*}Samples listed within the 'Other code' category had < 50 chickens from the processor sampled within the study. A <u>list of approved premises codes</u> can be found on the FSA website.

4.0 Discussion

4.1 Survey results

In this survey data set, *Campylobacter* spp. was detected in 54 % of fresh whole UK produced chicken at retail whilst 6 % (95 % CI = 5 to 7 %) of samples had >1000 cfu of campylobacters per g skin. In the previous survey year (PHE 2017), 61.3 % of chickens were contaminated and 11 % (95 % CI = 10 to 13 %) had >1000 cfu per g *Campylobacter* spp.. Very similar results were reported by the FSA based on a subset of the samples examined for this study (FSA 2017; 3980 that contained a minimum of 5 g neck-skin and results adjusted according to market share) showing 6.5 % of chickens with high levels of Campylobacter and 54.0 % positive for Campylobacter. Together this suggests that there is evidence of a significant reduction in contamination over the survey years.

Compared against the industry average, the group of smaller retail shops (including butchers) had the highest proportion of highly contaminated chickens. It may be reasonable to hypothesise that this could relate to various 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. Nevertheless, statistical analysis demonstrated that neither access to range during rearing, chicken weight at sale, days of shelf-life remaining, or season could explain this significant difference between these types of shops compared to the major retailer stores. Further studies would be needed to provide a more comprehensive understanding of the extent to which different processors can explain the observed difference in contamination. The logistic regression analysis found no effect of the survey quarter. However, in the current analysis, summer months were split into different quarters and further analysis would be needed to determine how this relates to the findings from the previous two survey years where the highest proportion of highly contaminated chickens was detected in the summer months.

There was significant 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 precluded an investigation of approval number in the logistic regression analyses. For example, approval number 5007 exclusively supplied the group of smaller retail shops and some butchers. 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 second survey year (PHE 2017).

From the majority of chicken skin samples (87.7 %) *C. jejuni* (only) was isolated while *C. coli* (only), was identified in 10.2 % of samples. A very similar species distribution was found in the previous survey years although in the previous survey years slightly lower proportions of *C. jejuni* were found (PHE 2015, PHE 2016). 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.

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 at retail sale in the UK that are contaminated with a high level of campylobacters has decreased considerably. However, the proportion of highly contaminated chickens from smaller retail shops remains high and suggests that more needs to be done to achieve better control of *Campylobacter* spp. in this part of the sector.

Overall, however, the data from this and the previous survey years has demonstrated a significant decline in the level of highly contaminated fresh whole UK retail chicken. The FSA has indicated that the average retail proxy for the proportion of highly contaminated retail chickens should be less than 7 % and continued monitoring can demonstrate that this decline is sustained.

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 89.9 per 100,000 in 2016. The rate of reported *Campylobacter* infections in England has decreased to the lowest rate reported since 2006, and remains below the rate observed in Wales and Scotland (Table 13).

Northern Ireland continues to report rates lower than the rest of the UK while Wales cases has continued to report the highest rates of infection (Table 13). The rate of reported *Campylobacter* infections in both Scotland and Wales have declined since 2014.

Table 13. Number and rate* of reported campylobacter infections in the United Kingdom and by country per 100,000 population, 2006-2016.

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
2016	48884	88.4	3497	112.3	5294	98.0	1258	67.6	58933	89.8

^{*}Rates were calculated based on the ONS 2016 mid-year population estimates

4.3 Conclusions

- The proportion of fresh whole chicken on retail sale in the UK that are contaminated with the highest level of campylobacter has on average, decreased considerably, but remains high for chickens from smaller retail shops.
- 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 of 18 % between 2014 and 2016. This reduction is most pronounced in England. Continued monitoring will be required to understand to what extent any decline maybe sustained.

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). Scientific 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://webarchive.nationalarchives.gov.uk/20180411152125tf /https://www.food.gov.uk/multimedia/pdfs/campsalmsurvey.pdf

Food Standards Agency (2009). FSA report for the UK survey of *Campylobacter* and *Salmonella* contamination of fresh chicken at retail sale. FSA Project B18025. http://webarchive.nationalarchives.gov.uk/20131206121901/http://food.gov.uk/multimedia/pdfs/fsis0409.pdf

Food Standards Agency (2010). The joint government and industry target to reduce campylobacter in UK produced chickens by 2015. Available at: http://webarchive.nationalarchives.gov.uk/20180411152125tf /https://www.food.gov.uk/sites/default/files/multimedia/pdfs/campytarget.pdf

Food Standards Agency (2013) Open Board – 11 September 2013 A refreshed strategy to reduce campylobacteriosis from poultry. Available at: http://webarchive.nationalarchives.gov.uk/20150809120004/http://www.food.gov.uk/sites/default/files/multimedia/pdfs/board/board-papers-2013/fsa-130904.pdf

Food Standards Agency (2015a). ACT: Acting on *Campylobacter* Together http://webarchive.nationalarchives.gov.uk/20160407013005/https://www.food.gov.uk/news-updates/campaigns/campylobacter

Food Standards Agency (2016). A UK wide microbiological survey of *Campylobacter* contamination in fresh whole chilled chickens at retail sale (Year 3/4) https://www.food.gov.uk/sites/default/files/retail_survey_protocol_year3.pdf. (Last accessed 22 January 2018).

Food Standards Agency (2015c). FSA Board meeting 15 July 2015: Update on the *Campylobacter* Campaign

http://webarchive.nationalarchives.gov.uk/20160407234941/https:/www.food.gov.uk/sites/default/files/fsa150705.pdf

Food Standards Agency (2015d). *Campylobacter* survey: cumulative results from the full 12 months (Q1 - Q4). Available at:

http://webarchive.nationalarchives.gov.uk/20160407023310/http://www.food.gov.uk/science/microbiology/campylobacterevidenceprogramme/retail-survey#toc-1

Food Standards Agency (2017). Campylobacter contamination in fresh whole chilled UK-produced chickens at retail: the final results from Year 3 (August 2016 to July 2017).

http://webarchive.nationalarchives.gov.uk/20180411173208/https:/www.food.gov.uk/sites/default/files/campyretailsurveyjul2017.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 2018)

Public Health England (2017). A microbiological survey of Campylobacter contamination in fresh whole UK-produced chilled chickens at retail sale. Year 2 Report. FSA Project FS102121.

https://www.food.gov.uk/sites/default/files/fsa-project-fs102121-year-2-report.pdf. (Last accessed 22 January 2018)

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.