



Summary

This pilot study has established methods for sampling and enumerating campylobacters in samples from fresh whole retail chickens. Enumeration using direct plating with a detection limit = 10 cells per g neck-skin or per swab-sample was used to examine retail chickens sampled from all major UK retailers according to market-share. For these pilot samples 22.1 % of samples had > 1000 cfu per g neck skin and 1.5 % of outer packaging samples had between 100 and 1374 cfu of *Campylobacter* per outer packaging sample. In conclusion the sampling and testing methods developed and employed in this pilot study were considered to provide a suitable basis on which to develop a statistically representative survey of whole UK-produced fresh retail chicken.

Background

Campylobacter species, especially *C. 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 GP consultations annually in the UK. Source-attribution studies, outbreak investigations and case-control reports all incriminate chicken meat as the key food-borne vehicle for *Campylobacter* infection. Cross contamination from poultry is believed to be an important transmission route.

The UK Food Standards Agency (FSA) has agreed a target with industry to reduce *Campylobacter* contamination in raw chicken as a measure of the effectiveness of the Agency's *Campylobacter* Risk Management Programme. The target is for a reduction in 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, from a 2008 baseline of 27 % to 10 % by 2015. The baseline was determined in 2008 using data obtained as part of an EU survey of *Campylobacter* on broiler carcasses where overall 87 % of the UK-produced chickens (testing ~ 400 carcasses) were positive for *Campylobacter* spp.. In March 2012 the FSA put in place a new ongoing representative UK monitoring programme at post-chill. The FSA is completing a review, with stakeholders, of the joint *Campylobacter* reduction target that was agreed in 2010, which has incorporated new data.

Packaging of raw chicken has been identified as a possible source of *Campylobacter* infection and in two previous small studies a prevalence of contamination of the outer packaging of ~ 6 % was reported. 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 handling of such packaging before opening. However, there is a lack of information on the *Campylobacter* levels present on outer packaging and how these levels relate to those on the chicken within the packaging. There is very limited information on the extent to which different swabbing methods may recover *Campylobacter* cells from outer packaging samples. The work described in the enclosed Annex I presents the extent to which *Campylobacter* cells could be recovered from a sponge-type swab using different swab wetting agents. Surfactants like Tween 80 minimize hydrophobic interactions between microbes and surfaces and may help release cells. The effect of adding this agent was also determined.

Examination of chicken samples in previous surveys have used a neck-skin sample as a representative sample of the chicken, however, there is little evidence to support how this sample may be best prepared. The work described in the enclosed Annex II evaluated counts obtained from chicken neck-skin samples from two different primary dilutions (1:2 and 1:9). In addition the detection from outer packaging swab samples using enrichment or direct plating was compared.



The methods determined in the development work (Annex I+II) were then used to examine numbers of campylobacters in further chicken samples collected for this pilot survey.

Objectives

This pilot survey project aimed to establish sampling and testing methods for determining levels of *Campylobacter* spp. on whole raw fresh chickens and their outer packaging and addressed the following objectives:

1. Determine levels of *Campylobacter* spp. in neck-skin samples from 400 whole UK-produced chilled chickens.
2. Determine levels of *Campylobacter* spp. on outer packaging samples from 400 whole UK-produced chilled chickens.
3. Undertake analysis of the distribution of counts of *Campylobacter* spp. from 400 chicken and their outer packaging samples.

Methods

Sampling

Samples were collected from retail premises in the UK and information gathered included temperature on sample receipt, approved premises code and use-by dates. Photographs of packaging and other sample details were recorded. No two chickens of the same product type, producer, production date and store were sampled.

Chickens sampled were:

- Pre-packed whole, chilled, raw, UK-produced standard, free range or organic chickens.
- Contained in a package which was unopened and undamaged.
- NOT frozen.
- NOT basted, herbed, stuffed marinated or otherwise modified.

Chickens were sampled for both the development phase and subsequent full pilot work according to a sampling plan aimed at reflecting market share (Table 1).

Table 1 Number of chickens to be obtained from each retailer.

Retailer	Development work		Final pilot protocol	
	E&W		E&W	NI + Scotland
ASDA	15		42	7
M&S	1		7	3
Morrisons	17		22	4
Sainsbury's	15		38	3
Tesco	30		78	15
Co-op	5		24	5
Waitrose	3		7	0
Others*	14		37	8
Totals	100		255	45



Sample examination

The samples examined as part of the development phase were prepared as follows (see also Appendix I):

Outer packaging sample

The wrapped chicken was placed on a disinfected plastic tray using disposable gloves. Maximum Recovery Diluent (MRD e.g. Oxoid CM0733; 10 ml) was added to a SpongeSicle™ swab. The entire surface of the packaging was then swabbed using this pre-wetted swab, swabbing areas twice using both sides of the swab.

Chicken skin

Wearing a fresh pair of disposable gloves, the chicken was removed from its wrapping, taking care not to allow the chicken skin to be contaminated. Using a sterile instrument and aseptic technique, skin from the neck (and breast if < 25 g neck skin available) was removed, avoiding fat, to make a 25 g test portion. If breast-skin was needed to achieve a 25 g sample the amount was recorded. BPW (225 ml) was added to the 25 g sample and this sample dilution was examined following stomaching by direct plating as described below.

*Enumeration of *Campylobacter* spp.*

Campylobacter spp. were enumerated by the surface plate method as described in the PHE Method - Detection and enumeration of *Campylobacter* spp.: F21. This method is partly based ISO/TS 10272-2:2006 Microbiology of food and animal feeding stuffs -- Horizontal method for detection and enumeration of *Campylobacter* spp. -- Part 2: Colony-count technique. According to this method the number of CFU of campylobacters was determined by plating modified Charcoal Cefoperazone Deoxycholate Agar (mCCDA). One ml of the outer packaging swab liquid was plated onto 3 pre-dried mCCDA plates. One ml of the chicken skin sample homogenate was plated onto 3 mCCDA plates. Additionally for chicken skin samples two further 10-fold dilutions were plated. The mCCDA plates were then incubated in a microaerophilic atmosphere at $41.5 \pm 1^\circ\text{C}$ for 44 ± 4 hours.

Following incubation, typical colonies on mCCDA were counted (e.g. 1-2 mm in diameter, greyish, often a little translucent, flat and moist colonies, sometimes with a metallic sheen). Five typical colonies (or fewer if less present) were selected from each presumptive positive sample and inoculated onto Columbia Blood Agar (containing 5 % (v/v) defibrinated sheep blood). Positive oxidase reaction, absence of growth under aerobic conditions and growth following incubation in a microaerophilic atmosphere (at 41.5°C for 24 – 48 h) was confirmed. Typical *Campylobacter* cell morphology (small, slim, curved or spiral, Gram-negative rods) was confirmed and additional confirmatory tests (e.g. latex agglutination) were performed as required.

Isolates of *Campylobacter* spp. were sent to the Gastrointestinal Bacterial Reference Unit at the PHE Microbiology Services, Colindale in London for further characterisation.

Results

*Campylobacter*s were not detected (detection limit = 10 cells per outer-packaging sample) in the large majority of outer-packaging samples examined in the pilot study (Figure 1; total of 412 samples). In 7.8 % of samples campylobacters were detected from the outer-packaging samples mostly at low levels but in 1.5 % of samples between 100 and 1374 campylobacter cfu per sample were detected.

*Campylobacter*s were detected in the large majority of chicken skin samples (81.1 %) and 22.1 % of the chicken skin samples had counts above 1000 cfu per g chicken skin (Figure 2). The highest count detected was 58,000 cfu of *Campylobacter* per g chicken skin.

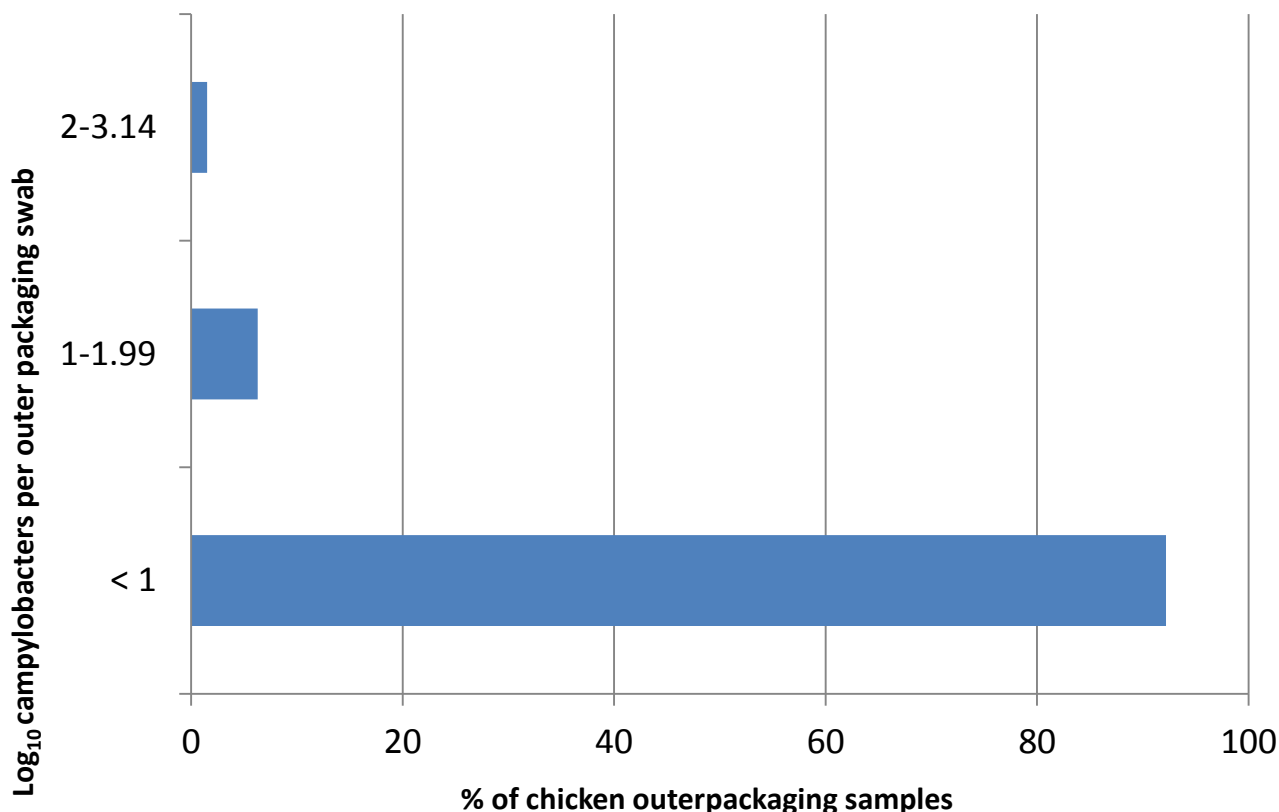


Figure 1 Distribution of the number of cfu of *Campylobacter* spp. per outer packaging swab.

Campylobacter jejuni (284) and *C. coli* (52) strains were tested for sensitivity to antimicrobials including Ampicillin (A), Chloramphenicol (C), Ciprofloxacin (Cp), Erythromycin (E), Gentamicin (G), Kanamycin (K), Nalidixic Acid (Nx), Neomycin (Ne) and Tetracycline (T) (Table 2). A higher proportion of both *C. jejuni* (40 %) and *C. coli* (54 %) strains were resistant to Cp compared to the 2007 retail survey (Anon. 2009). Only one (*C. jejuni*) strain was resistant to erythromycin. There were 66 strains that were sensitive to all the antibiotics tested as listed in Table 2.

Table 2 Antimicrobial resistance for *Campylobacter jejuni* and *C. coli* isolates from retail fresh whole chicken.

Antibiotic (abbreviation)	Breakpoint concentration used (mg/l)	Number of resistant strains (% of total)	Number of resistant <i>C. jejuni</i> strains/284 (%)	Number of resistant <i>C. coli</i> strains/52 (%)
Ampicillin (A)	32	230 (68)	202 (71)	28 (54)
Chloramphenicol (C)	8	1 (0.3)	1 (0.4)	0 (< 1.9)
Ciprofloxacin (Cp)	1	142 (42)	114 (40)	28 (54)
Erythromycin (E)	4	1 (0.3)	1 (0.4)	0 (< 1.9)
Gentamycin (G)	4	0 (< 0.3)	0 (< 0.3)	0 (< 1.9)
Kanamycin (K)	16	2 (0.6)	0 (< 0.3)	2 (4)
Nalidixic acid (Nx)	16	143 (43)	115 (40)	28 (54)
Neomycin (Ne)	8	2 (0.6)	0 (< 0.3)	2 (4)
Tetracycline (T)	128	158 (47)	137 (48)	21 (40)

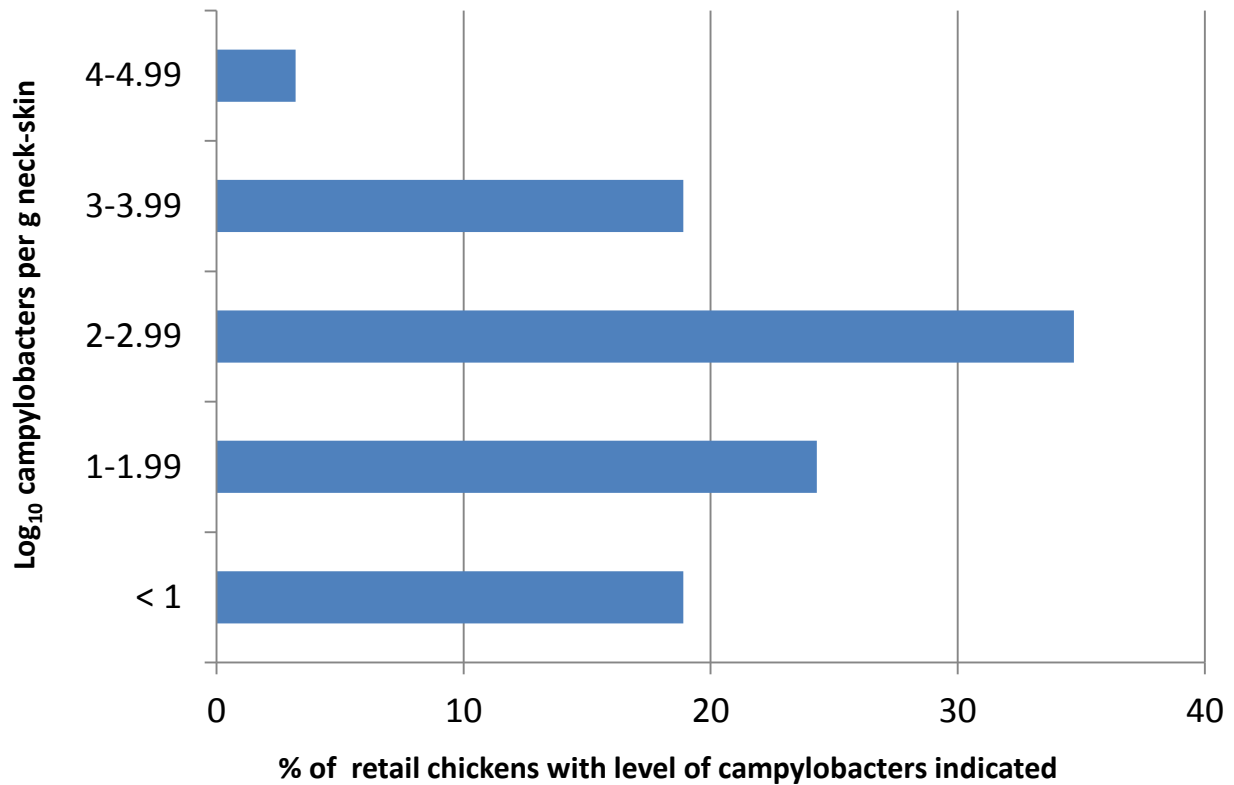


Figure 2 Distribution of the number of cfu of *Campylobacter* spp. per g chicken neck-skin (1:9 dilutions results only shown).

Conclusions

The method protocol created in the development phase of this pilot study was used to examine further chicken samples to establish whether it was suitable for the planned full retail survey. Taking into account the need to obtain reliable and accurate enumeration data while maintaining a reasonable detection limit the most practical method for examining the chickens for *Campylobacter* contamination was a 1:9 dilution of a chicken neck-skin sample. The use of a direct plating method for examining outer packaging swab samples did not result in isolation rates much different to those reported in previous studies employing enrichment methods (Jorgensen et al. 2002; Bolton et al. 1999). As expected a relatively low proportion of outer packaging samples tested positive for campylobacters and all these were at low levels. The level of erythromycin resistance was low while resistance to ciprofloxacin was similar to the previous survey.



References

Anonymous

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ANNEX I

Swab method development



Background

Campylobacter species, especially *C. jejuni* and *C. coli*, are the main cause of human bacterial gastroenteritis in the developed world and the number of cases in the UK are increasing, causing over half a million cases and 80,000 GP consultations annually. Source-attribution studies, outbreak investigations and case-control reports all incriminate chicken meat as the key food-borne vehicle for *Campylobacter* infection. Cross contamination from poultry is believed to be an important transmission route.

Packaging of raw chicken has been identified as a possible source of infection and in two previous small studies a prevalence of contamination of the outer packaging of ~ 6 % was reported. The presence of *Campylobacter* spp. on the outer packaging of chicken packs raises concern as consumers would not expect products to be contaminated on the outside and would consequently do nothing to avoid cross-contamination.

However, there is a lack of information on levels of campylobacters than may be detected on outer packaging and very limited published information on the extent to which different swabbing methods may recover cells from swab samples.

The aim of the work described in this Annex I was to determine the extent to which *Campylobacter* cells could be recovered from a sponge-type swab using different swab wetting agents. Surfactants like Tween 80 minimize hydrophobic interactions between microbes and surfaces and may help release cells and the effect of adding this agent was also determined.

Methods and Results

Method development study (swab method)

Recovery of *Campylobacter* spp. from swabs was investigated using SpongeSicle™ swabs (a sponge type stick-swab contained in a sterile bag). For each experiment 9.9 ml of wetting agent was added to the dry swab in its bag. After 30 minutes *Campylobacter* cells (~10⁴-10⁵ *Campylobacter jejuni* (NCTC 11322) or *Campylobacter coli* (NCTC 11366) in 100 µl) were added. Each inoculum was prepared by suspending one colony from a Blood Agar (BA) plate (inoculated from a bead) into 1 ml MRD and this suspension was further diluted in MRD to achieve a concentration of ~10⁵-10⁶ CFU per ml MRD as determined by plating (see below).

The inoculated swab was held in its bag for 30 minutes and then stomached for 30 s. The swab liquid was then squeezed out into a corner of the bag and sampled using a sterile pastette. *Campylobacter*s were then enumerated in this liquid (examining also one dilution thereof) to determine the number of CFU of campylobacters released (into the liquid) from the swab.

Two swab wetting agent were examined:

Buffered Peptone Water Broth (BPW e.g. Oxoid CM0509) and
Maximum Recovery Diluent (MRD e.g. Oxoid CM0733)

Addition of 0.01 % (w/v) Tween 80* (Polysorbate 80 C.A.S. 9005-65-6) was also tested.

The number of CFU of campylobacters was determined by plating onto duplicate blood agar (BA) (and modified Charcoal Cefoperazone Deoxycholate Agar (mCCDA) in some experiments) and plates were incubated for 48 h at 41.5 °C in a microaerobic atmosphere. Colonies were then counted and confirmed according to ISO/TS 10272-2:2006 'Microbiology of food and animal feeding stuffs — Horizontal method for detection and enumeration of *Campylobacter* spp. Part 2: Colony-count technique'.



The proportion of cells recovered with each type of swab wetting agent was calculated by subtracting the log₁₀ number of cells released from the sponge swab from the log₁₀ number of cells originally inoculated onto the swab.

Table 1 Proportion of cfu of campylobacters recovered in relation to wetting agent.

Wetting agent	Average ^a (log ₁₀ inoculum - log ₁₀ released); SD	
	<i>Campylobacter</i> strain	
	<i>C. jejuni</i> NCTC 11322	<i>C. coli</i> NCTC 11366
BPW	-0.0865 ; 0.105	-0.1423 ; 0.174
MRD	-0.0397 ; 0.096	-0.0828 ; 0.094
MRD+0.01 % Tween	0.0576 ; 0.113	0.0035 ; 0.137

^a Each average was calculated from 3 to 5 experiments per wetting agent/strain.

There was no significant difference in proportion of *C. jejuni* cells recovered from swabs using MRD compared to using BPW as the swab wetting agent (the probability associated with a Student's paired t-Test, with a two-tailed distribution = 0.51).

There was no significant difference in proportion of *C. coli* cells recovered from swabs using MRD compared to using BPW as swab wetting agent (the probability associated with a Student's paired t-Test, with a two-tailed distribution = 0.93).

There was no significant difference in the proportion of *C. jejuni* cells recovered from swabs using MRD compared to using MRD with 0.01 % Tween as swab wetting agent (the probability associated with a Student's paired t-Test, with a two-tailed distribution = 0.14).

There was no significant difference in the proportion of *C. coli* cells recovered from swabs using MRD compared to using MRD with 0.01 % Tween as swab wetting agent (the probability associated with a Student's paired t-Test, with a two-tailed distribution = 0.12).

A paired T-test demonstrated that there was no overall significant difference (P = 0.17) between the number of cells inoculated onto the swab and the number of cells recovered, regardless of wetting agent, demonstrating that the recovery from the swab type using either BPW or MRD was effective.

The use of only 5 ml wetting agent proved impractical as this amount of liquid was insufficient for assuring the entire sponge could be wetted/washed thoroughly.

Conclusions

Method development study (swab method)

There was consistent recovery of two *Campylobacter* species (*C. jejuni* and *C. coli*) from the SpongeSicle™ swab with no significant difference between the number of cells initially added to the swab and the number of cells subsequently released. This type of swab is, therefore considered suitable for use when recovering *Campylobacter* cells from packaging surfaces. While it cannot be ruled out that other swab types may be equally suitable the SpongeSicle swab was considered appropriate for the purposes of this project. There was no significant difference in the number of cells recovered from swabs between using MRD or MRD with 0.01 % Tween as a swab wetting agent. It is possible that Tween may be detrimental to damaged *Campylobacter* cells and for this reason addition of Tween in the swab meth can be omitted. It would require further work to ascertain to what extent Tween could have any detrimental effect on physiologically stressed *Campylobacter* cells (it is likely that cells on outer packaging would be stressed) and whether this could negate any possible benefit from improved removal of cells adhered to surfaces.

In conclusion, the swab method should be included in the protocol for examining the outside packaging of the retail chickens in the pilot study employing SpongeSicle™ swabs pre-wetted with 10 ml MRD.



ANNEX II

Skin and swab sample method development



Background

Campylobacter species, especially *C. jejuni* and *C. coli*, are the main cause of human bacterial gastroenteritis in the developed world and the number of cases in the UK are increasing, causing over half a million cases and 80,000 GP consultations annually. Source-attribution studies, outbreak investigations and case-control reports all incriminate chicken meat as the key food-borne vehicle for *Campylobacter* infection. Cross contamination from poultry is believed to be an important transmission route.

The UK Food Standards Agency (FSA) has agreed a target with industry to reduce *Campylobacter* contamination in raw chicken as a measure of the effectiveness of the Agency's *Campylobacter* Risk Management Programme. This pilot study is contributing to the evidence base towards reducing *Campylobacter* contamination on raw chicken.

Packaging of raw chicken has been identified as a possible source of infection and in two previous small studies a prevalence of contamination of the outer packaging of ~ 6 % was reported. The presence of *Campylobacter* spp. on the outer packaging of chicken packs raised concern as consumers would not expect products to be contaminated on the outside and would consequently do nothing to avoid cross-contamination. However, there is a lack of information on the levels detected on outer packaging and how these levels relate to levels on the chicken it contains.

The aim of the work described in this report was to evaluate the counts obtained from chicken skin samples from two initial BPW-dilutions (1:2 and 1:9) and to compare detection using enrichment or direct plating from outer packaging swab samples.

Methods

This development work aimed to establish sampling and testing methods for determining levels of *Campylobacter* spp. on whole chickens and their outer packaging and addressed the following objectives:

1. Determine levels of *Campylobacter* spp. in skin samples using two dilutions from 100 whole UK-produced chilled chickens
2. Comparing detection of *Campylobacter* spp. on the outer packaging samples obtained from 100 chickens using detection by enrichment and direct plating.
3. Undertake analysis of the distribution of counts of *Campylobacter* spp. from 100 chicken and their outer packaging samples

Samples were collected in from retail premises in England and testing performed by the Public Health England Food, Water and Environmental Porton and London laboratories.

Additional information gathered included temperature on collection, approved premises code and use-by dates. Photographs of packaging and other sample details were recorded. No two chickens of the same product type, producer, production date and store were sampled.



Chickens sampled were:

- Pre-packed whole raw chilled UK-produced standard, free range or organic chickens.
- Contained in a package which was unopened and undamaged.
- NOT frozen.
- NOT basted, herbed, stuffed marinated or otherwise modified.

The chickens were obtained from retailers in a manner that reflected market share.

Table 1 Number of chickens obtained from each retailer.

Development work	
Retailer	E&W
ASDA	15
M&S	1
Morrisons	17
Sainsbury's	15
Tesco	30
Co-op	5
Waitrose	3
Others*	14
Totals	100

Sample examination

Packaging

The wrapped chicken was placed on a disinfected plastic tray using disposable gloves. Maximum Recovery Diluent (MRD e.g. Oxoid CM0733) (10 ml) was added to a SpongeSicle™ swab. The entire surface of the packaging was swabbed using this pre-wetted swab, swabbing areas twice using both side of the swab.

Chicken skin

Wearing a fresh pair of disposable gloves, the chicken was removed from its wrapping, taking care not to allow the chicken skin to be contaminated. Using a sterile instrument and aseptic technique, skin from the neck (and breast if < 25 g neck skin available) avoiding fat was removed to make a 25 g test portion.

If breast-skin was needed to achieve a 25 g sample the amount was recorded.

Recovery of campylobacter cells from chicken skin samples was examined for two proportions of skin:BPW-diluent (1:2 and 1:9).

BPW (50 ml) was added to the 25 g sample and this 1:2 sample dilution was examined following stomaching by direct plating as described below. Then an additional 175 ml of BPW was added to the 1:2 skin-BPW homogenate resulting in a 1:9 sample dilution and following stomaching this was also examined for campylobacters as carried out for the 1:2 homogenate.

Enumeration of *Campylobacter* spp.

Campylobacter spp. were enumerated by the surface plate method as described in the HPA Methods - Detection and enumeration of *Campylobacter* spp.: F21. This method is based on ISO/TS 10272-2:2006 Microbiology of food and animal feeding stuffs -- Horizontal method for detection and enumeration of *Campylobacter* spp. -- Part 2: Colony-count technique. According to this method the number of CFU of campylobacters was determined by plating modified Charcoal Cefoperazone Deoxycholate Agar (mCCDA).



One ml of the outer packaging swab liquid was plated onto 3 pre-dried mCCDA plates. One ml of the chicken skin sample homogenate was plated onto 3 mCCDA plates. Additionally for chicken skin samples two further 10-fold dilutions were plated.

The mCCDA plates were then incubated in a microaerophilic atmosphere at $41.5 \pm 1^\circ\text{C}$ for 44 ± 4 hours.

Following incubation, typical colonies on mCCDA were counted (e.g. 1-2 mm in diameter, greyish, often a little translucent, flat and moist colonies, sometimes with a metallic sheen). Five typical colonies (or fewer if less present) were selected from each presumptive positive sample and inoculated onto Columbia Blood Agar (containing 5 % (v/v) defibrinated sheep blood). Positive oxidase reaction, absence of growth under aerobic conditions and growth following incubation in a microaerophilic atmosphere (at 41.5°C for 24 – 48 h) was confirmed. Typical campylobacter cell morphology (small, slim, curved or spiral, Gram-negative rods) was confirmed and additional confirmatory tests (e.g. latex agglutination) were performed as required.

Isolates of *Campylobacter* spp. were sent to the Gastrointestinal Bacterial Reference Unit at the PHE Microbiology Services, Colindale in London for further characterisation.

Enrichment of *Campylobacter* spp.

Campylobacter spp. were examined by enrichment from the outer packaging swabs as described in the PHE method: Detection and enumeration of *Campylobacter* spp.: F21. This method is based on BS EN ISO 10272-1. According to this method 81 ml Bolton broth was added to the 9 ml swab liquid remaining in the bag and the bag was incubated with a small headspace. After incubation the enriched samples was streaked onto mCCDA plates that were incubated in a microaerophilic atmosphere at $41.5 \pm 1^\circ\text{C}$ for 44 ± 4 hours. Suspect colonies were then confirmed as described above for the enumeration method.

Results

*Campylobacter*s were detected in 12 outer-packaging swab samples by both or either of the detection methods employed (Table 1). Detection using the enrichment method detected campylobacters in 4 samples compared to detection in 7 samples using the direct plating method (Table 1), however no statistical significant difference was found ($P = 0.34$; Fishers exact test). The number of cfu of campylobacters detected by direct plating ranged from 1 to 12 cfu in the 1 ml of swab suspension plated, resulting in values ranging from 1.0 to 2.1 \log_{10} cfu detected per outer packaging swab.

Table 1 Number of occasions where campylobacters were detected (+) or not detected (-) in swab samples collected from outer packaging of whole retail chicken packs using enrichment or direct plating.

	Enrichment	
	+	-
Direct plating	+	7
	-	90

For the development phase of the pilot study 102 whole retail chickens were examined for campylobacters. *Campylobacter*s were detected in 86 of neck/(breast)- skin samples from both or either of the 1:2 and 1:9 dilutions tested (Table 2). Cohens kappa test suggested good detection agreement between the two methods ($\text{Kappa} = 0.68$). About a quarter of the chickens had counts above 1000 cells per g sample (using the highest count obtained from either dilution; Figure 1).



Table 2 Number of occasions where campylobacters were detected (+) or not detected (-) in skin samples of raw, fresh whole retail chickens from a 1:2 or 1:9 dilution.

	1:2 dilution		
		+	-
1:9 dilution	+	75*	4
	-	7	16

*Counting was not possible for 3 of the 1:2 dilution plates.

For some samples $> 0.5 \log_{10}$ difference between the two sample dilutions was recorded with 8 of the 1:9 dilution counts exceeding the 1:2 dilution counts and 4 of the 1:2 dilution counts exceeding the 1:9 dilution counts (Figure 2). Results from the 1:2 dilution plates, detected campylobacters from 3 more samples but in 3 of the 1:2 dilution samples no accurate count was obtained due to swarming on the plates.

On balance if a preference is for accurate and reliable counts of > 10 per g rather than increasing the detection rate of very low counts this data would seem to support the use the 1:9 dilution.

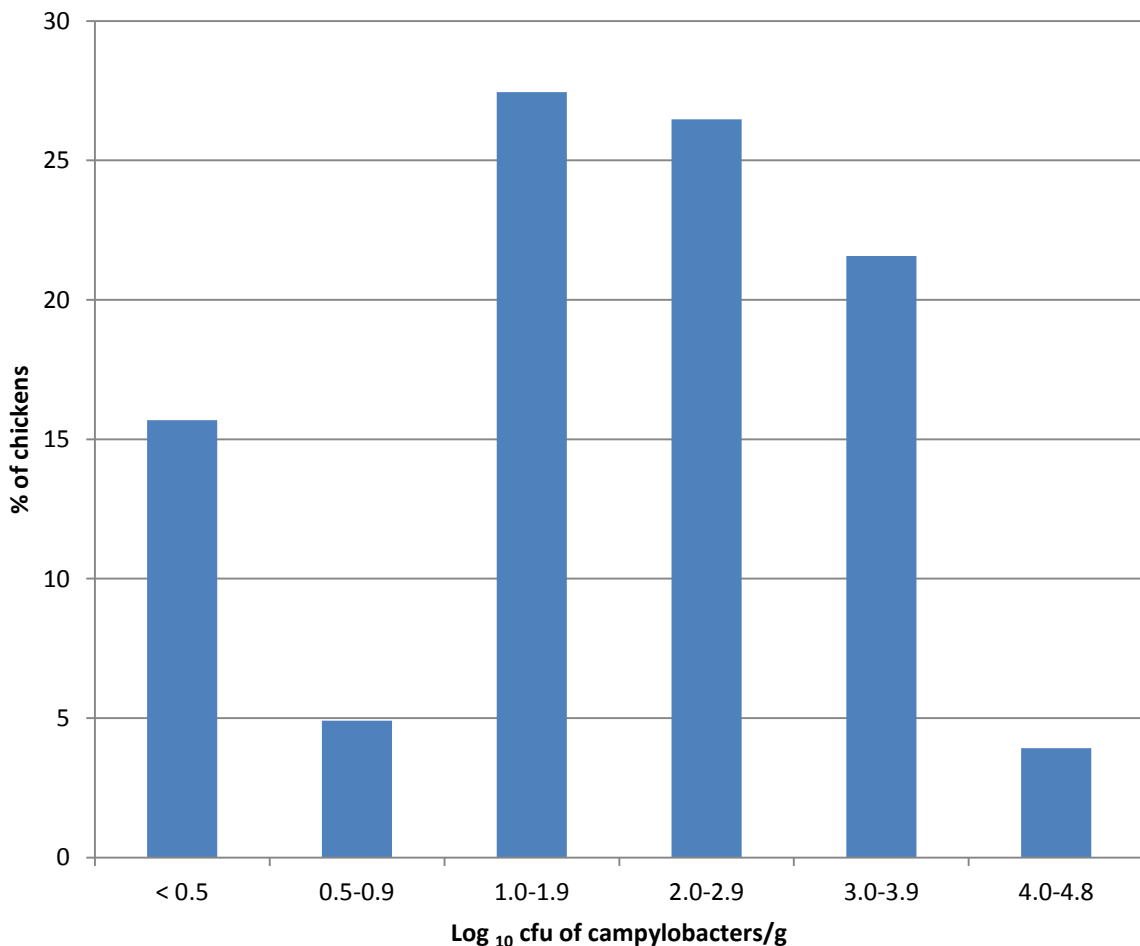


Figure 1 Distribution of the number of cfu of *Campylobacter* spp. per g chicken sample obtained from either the 1:2 or 1:9 sample dilution plated (highest count shown; see Figure 2 for the extent of agreement between testing the 1:2 and 1:9 level).



Campylobacter jejuni (76) and *C. coli* (6) were tested for sensitivity to antimicrobials including Ampicillin (A), Chloramphenicol (C), Ciprofloxacin (Cp), Erythromycin (E), Gentamicin (G), Kanamycin (K), Nalidixic Acid (Nx), Neomycin (Ne) and Tetracycline (T). None of the strains were resistant to E, G, K or Ne but 26 (32%) were resistant to Cp.

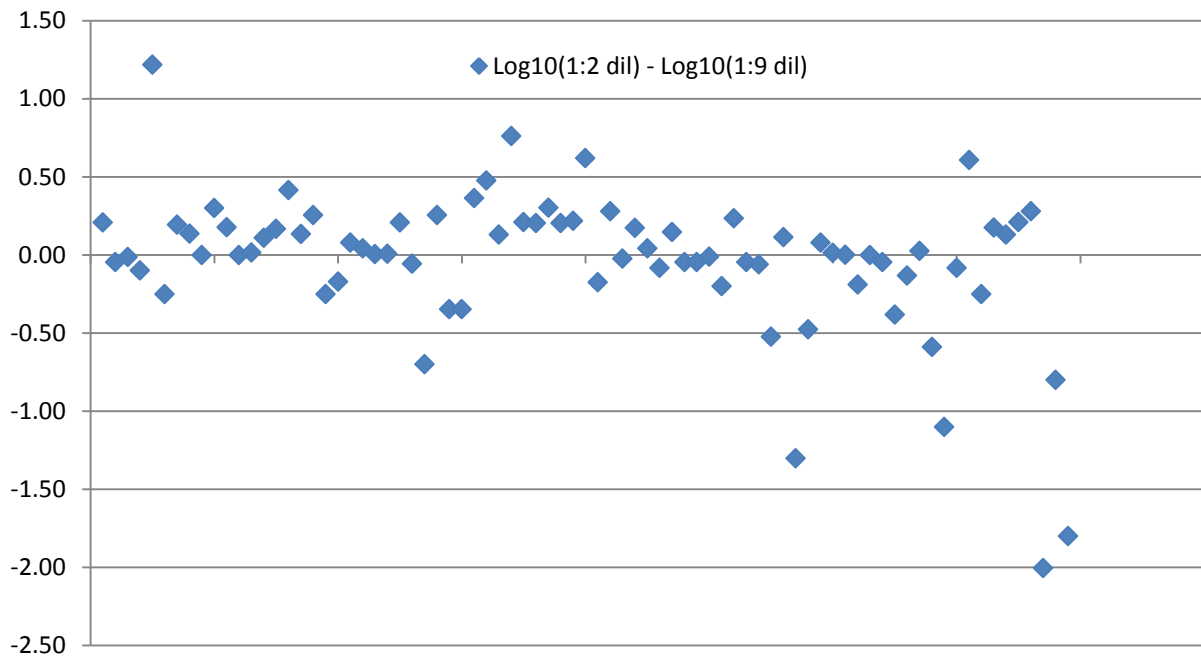


Figure 2 Comparison of the number of cfu of *Campylobacter* spp. per g chicken skin obtained from 1:2 and 1:9 dilutions of the same sample. Values of zero means there was no difference between the 1:2 and the 1:9 dilutions tested – values above zero suggest the count was higher in the 1:2 dilution while values below zero suggest the count was higher in the 1:9 dilution tested.

Conclusions

Taking into account the need to obtain reliable and accurate enumeration data while not missing positive samples above a reasonable detection limit the most practical method for examining the remaining 300 chickens in the pilot study was to employ the 1:9 dilution method. As expected a low proportion of outer packaging swab samples were positive for *Campylobacter* with direct plating providing slightly better detection than when enumeration was used.