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The prevalence of *Campylobacter* spp. in broiler flocks and on broiler carcases, and the risks associated with highly contaminated carcases

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SUMMARY

A baseline survey on the prevalence of *Campylobacter* spp. in broiler flocks and *Campylobacter* spp. on broiler carcases in the UK was performed in 2008 in accordance with Commission Decision 2007/516/EC. Pooled caecal contents from each randomly selected slaughter batch, and neck and breast skin from a single carcase were examined for *Campylobacter* spp. The prevalence of *Campylobacter* in the caeca of broiler batches was 75·8% (303/400) compared to 87·3% (349/400) on broiler carcases. Overall, 27·3% of the carcases were found to be highly contaminated with *Campylobacter* ($\geqslant$1000 c.f.u./g). Slaughter in the summer months (June, July, August) [odds ratio (OR) 3·50], previous partial depopulation of the flock (OR 3·37), and an increased mortality at 14 days ($\geqslant$1·25% to $<$1·75%) (OR 2·54) were identified as significant risk factors for the most heavily *Campylobacter*-contaminated carcases. Four poultry companies and farm location were also found to be significantly associated with highly contaminated carcases.

Key words: Broiler, *Campylobacter*, carcase, contamination, risk factors.

INTRODUCTION

In order to establish baseline and comparable data for all Member States a series of baseline prevalence surveys have been conducted within the European Union (EU), including *Salmonella* in laying-hen flocks [1] and *Salmonella* in broiler flocks [2]. In 2008, under Decision 2007/516/EC, an EU-wide baseline survey was performed to determine the prevalence of *Campylobacter* in broiler batches and *Campylobacter* and *Salmonella* on broiler carcases. This is the first baseline survey to include *Campylobacter* and broiler carcases intended for human consumption.

Campylobacter infections in people continue to be a major public health concern in the UK and Europe. *Campylobacter* is the most frequently reported infectious intestinal disease in people in the EU and infections are associated with considerable morbidity and economic loss [3–5]. A rise in the number of confirmed cases of human campylobacteriosis has been reported in the EU [6]. In 2010 in England and Wales alone, there were 62 684 laboratory-confirmed human cases of campylobacteriosis, a rise of 8·5% compared to 2009 [7]. Under-ascertainment of infectious intestinal disease is well recognized, hence the true population burden is likely to be far greater [3, 8].

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The handling or consumption of undercooked chicken is considered to be a major risk factor for *Campylobacter* infections in humans [9–11]. Broiler flocks are considered to be a natural host for *Campylobacter* spp. and colonized birds can carry a very high *Campylobacter* load in their gastrointestinal tract. Broiler flocks are frequently colonized with *Campylobacter* spp., the prevalence of *Campylobacter* in UK broiler flocks has been previously estimated at 34·2% [12], 42% [13] and 81·6% [14] and studies have found *Campylobacter* on 65% [15] and 91% [16] of chicken carcases sampled at retail sale.

The control of *Campylobacter* has primarily focused on reducing the presence and numbers of *Campylobacter* in poultry flocks. Indeed previous research has estimated that a 2-log reduction in the number of campylobacters on chicken carcases would lead to a 30-fold reduction in human cases associated with the consumption of chicken [17]. Procedures to control or limit the contamination of poultry carcases with *Campylobacter* during processing are available [18]; however, they are limited by their practicality, restrictions under EU food legislation or acceptability to consumers [19].

A voluntary industry target has recently been agreed with the UK government to reduce *Campylobacter* in UK-produced chickens by 2015 [20]. The target is based on *Campylobacter* counts (enumeration) on carcases and aims to reduce the levels of the most highly contaminated chickens at the end of the slaughter process (post-chill). The target focuses on decreasing the proportion of birds in the most contaminated group [those with *Campylobacter* counts of >1000 colony-forming units per gram (c.f.u./g)]. In light of this new target we have examined the characteristics of the most contaminated slaughter batches to identify any risks associated with such contamination.

This paper reports the *Campylobacter* results from a UK-wide survey. The aims of the study were to estimate the prevalence of *Campylobacter* in the caeca of broiler flocks and the prevalence of *Campylobacter* on broiler carcases and to identify factors associated with the most heavily *Campylobacter*-contaminated carcases.

METHODS

Sampling plan

The survey was conducted over 12 months from January to December 2008. The survey design and sampling methods used conformed with the technical specifications annexed to Decision 2007/516/EC. To avoid seasonal bias sampling was distributed equally over the year.

Study population

The UK population for the survey was defined in December 2007, at this time 73 slaughterhouses operated under Regulation (EC) No. 853/2004, with a combined annual throughput of about 822 million chickens. This study recruited 36 slaughterhouses into the survey, with a combined throughput of 722 million chickens, representing 88% of the annual kill in the UK. Two of the 36 slaughterhouses recruited to the survey closed during 2008, slaughter batches that were due to be sampled from these slaughterhouses were assigned to the relevant slaughterhouses in their respective companies ensuring that the original estimate of 88% representation of annual kill in the UK was maintained.

Samples and schedules

The UK had a target sample size of 384 slaughter batches, as set by the EU, based on an expected prevalence of 50% with an accuracy of 5% and a confidence level of 95%. A slaughter batch was defined as a quantity of broilers that had been raised on the same farm premises in the same shed/enclosure and delivered to the abattoir in the same vehicle. In anticipation of non-responses and slaughter batch samples that did not meet the eligibility criteria for the survey, the sample size was increased to 451 slaughter batches to ensure the survey target was met.

Selection of the slaughterhouse for each sample was randomized and weighted; as such the probability of a high throughput abattoir being selected to sample was higher compared with a lower throughput plant. The procedure for selecting the random sample was set up in Microsoft® Access. The sampling day within each month was randomly chosen from the days the selected slaughterhouse was open. The individual slaughter batch to be sampled was randomly chosen from the total number of batches that the selected slaughterhouse processed daily, reserve batch numbers were also scheduled in case the first selected batch was not suitable for sampling. Samples scheduled for collection during the Christmas and Easter holiday periods were reallocated to other dates within the same month.

Of the 36 abattoirs participating in the survey, a total of 25 abattoirs, associated with 17 parent companies, were randomly selected to take part in the survey. The target of 32 eligible batches per month was met or exceeded for all months of the survey except for January and April when 31 eligible batches were sampled.

Sample collection

Staff trained in standard sampling procedures collected the samples. In England, Wales and Scotland samples were collected by the Meat Hygiene Service (MHS), an executive agency of the Food Standards Agency (FSA). Samples were collected in Northern Ireland by the Veterinary Public Health Unit of the Department of Agriculture and Rural Development (DARD).

From each randomly selected slaughter batch, 10 pairs of intact and full caeca were collected at random from the selected slaughter batch. Pairs of caeca were collected at evisceration, the first part of the slaughter batch was not sampled (to minimize risk of unknowingly including a bird from a different batch) and caeca from consecutive birds were not collected. A single carcase with neck skin was collected at random from the same slaughter batch. The carcase was collected post-chilling but pre-processing and was not taken from the first part of the slaughter batch (again to minimize the risk of unknowingly including a bird from a different batch).

All caeca and carcases were individually wrapped and then transported to the laboratory with minimum delay in an insulated shipping box that held samples at between 2 °C and 8 °C for up to 72 h.

Data collection

Each sample collected at the slaughterhouse was accompanied by a standardized data questionnaire completed by the sampler that collected information on attributes that could affect the *Campylobacter* status of the slaughter batch, including age of birds, farm name and address and flock mortality data. No data on processing variables were collected. A second standardized questionnaire was sent to the poultry company contact at the abattoir following sampling to obtain details on flock depopulation status. If no information was available on previous removal of birds from the house of the sampled slaughter batch, or if the information was unclear, the 'previous depopulation' status for that flock was considered unknown.

Eligibility criteria

Samples that were tested for *Campylobacter* and *Salmonella* within 80 h of collection and which included ten caeca samples and a carcase whose temperature was $\leqslant 8$ °C upon arrival at the laboratory were included in the analysis according to EU guidelines. Only slaughter batches raised as single flocks rather than mixed from more than one flock were eligible for inclusion in the analysis.

Of the 451 slaughter batches scheduled for sampling 445 batches were sampled and of these, 400 slaughter batches were eligible for inclusion in the survey. Of the 45 slaughter batches classed as ineligible, according to EU baseline survey criteria, most batches ($n=33$) were excluded because the samples had exceeded the 80-h deadline between sample collection and processing. A further nine slaughter batches were ineligible because the carcase sample was missing and the remaining three slaughter batches were ineligible because one batch was from multiple houses, one was not tested for *Salmonella* and one of the carcases had a temperature >8 °C.

Microbiological methods

The culture of caecal and carcase samples for *Campylobacter* was performed by the Food and Environmental Safety (FES) Department, VLA (GB) and by the Agri-Food and Biosciences Institute (AFBI), Stormont Veterinary Laboratory (NI).

Caeca – *Campylobacter* detection

The method used for the detection and speciation of *Campylobacter* spp. in caecal samples was in accordance with the technical specifications set out in Annex I of the Commission Decision 2007/516/EC. Briefly, the contents of ten caeca per slaughter batch (one caecum per bird) were removed and pooled into one composite sample. The pooled sample was streaked directly onto modified charcoal cefoperazone deoxycholate agar (mCCDA) (CM739 and supplement SR155, Oxoid, UK). Cultures were incubated in a microaerobic atmosphere (84% N_2/10% CO_2/6% O_2) at $41{\cdot}5 \pm 1$ °C and examined for typical *Campylobacter* colonies after 24–48 h.

Where possible, five colonies showing typical morphology for *Campylobacter* were taken from each mCCDA plate and subcultured onto 7% sheep blood agar (CM0055, Oxoid, 7% sheep blood and 1000 mg cyclohexamide/litre). All suspect cultures were confirmed as thermophilic *Campylobacter* spp. by colony morphology, cell morphology and motility, and catalase and oxidase activity and growth conditions in accord with ISO 10272-1:2006. A single *Campylobacter* spp. colony from each caecal sample was identified to species level by hippurate and indoxylacetate hydrolysis and the sensitivity profile to cephalothin and nalidixic acid according to ISO 10272-1:2006.

A slaughter batch was considered positive if at least one confirmed colony of *Campylobacter* spp. was isolated.

Carcase – *Campylobacter* detection

A 27-g portion of neck and breast skin from each carcase was added to 243 ml buffered peptone water (BPW) and then treated in a stomacher for 1 min to create the initial carcase suspension for further testing.

The detection of *Campylobacter* spp. from carcases was as detailed in the EC technical specifications (2007/516/EC) and in accord with ISO 10272-1:2006. Briefly, 10 ml initial carcase suspension was inoculated into 90 ml Bolton enrichment broth (CM0983 and supplement SR0183e, Oxoid and 5% lysed horse blood). The broth was incubated microaerobically at 37 ± 3 °C for 4–6 h followed by 41·5 °C for 44 h. Samples of broth culture were then plated onto mCCDA and Preston agar (CM0689 and supplement SR0117, Oxoid, and 5% lysed horse blood). Plates were then incubated and examined for *Campylobacter* as described for caecal culture above. All colonies confirmed as *Campylobacter* spp. were fully identified to species level as indicated above.

In this study a carcase sample was declared positive if at least one confirmed colony of *Campylobacter* spp. (from either mCCDA or Preston agar) was isolated.

Carcase – *Campylobacter* enumeration

The method used for the enumeration of *Campylobacter* spp. on carcases was as described in EC technical specifications (2007/516/EC) and ISO 10272:2006 (part 2) and ISO 7218:2007E. In brief, 10 ml of the initial carcase suspension was tenfold serially diluted in phosphate-buffered saline, 0·1 M (pH 7·1), until a 10^{-5} dilution was created. For each dilution (including initial carcase suspension), 0·1 ml was plated onto mCCDA. Additionally 1-ml aliquots of the initial carcase suspension were spread over a pair of large mCCDA plates (140 mm diameter) to obtain a limit of detection of 5 c.f.u./g. Plates were microaerobically incubated at 41·5 °C for 24–48 h. *Campylobacter* colonies were counted and confirmed as *Campylobacter* spp. according to ISO 10272-1:2006 and the final calculation and reporting of *Campylobacter* c.f.u./g of neck and breast skin were as described in ISO 7218:2007E. Briefly, suspect *Campylobacter* colonies were counted from the plate inoculated with the lowest dilution that contained between 10 and 150 colonies and from the plate inoculated with the consecutive dilution. When colonies were counted from 1-ml plates, only the two duplicate plates were counted. Up to 5 colonies per counted plate were confirmed as *Campylobacter* spp. Based on the percentage of confirmed campylobacters per plate, the counts were adjusted and a final count calculated. The final number of campylobacters present in the sample was calculated as a weighted mean from the two consecutive dilutions, using the formula:

$$N = \frac{\sum C}{V \times (1 \times n1) + (0{\cdot}1 \times n2) \times d}$$

where ΣC is the sum of the colonies on the two plates after adjustment; V is the volume of inoculum, $n1$ and $n2$ are the first and second dilution counted plates and d is the lowest dilution counted.

A result was valid if at least 10 *Campylobacter* spp. colonies were counted on a minimum of one plate. If the calculation was based on a count between four and nine *Campylobacter* spp. colonies on a plate, an estimated number of *Campylobacter* spp./g was reported. If the calculation was based on a count of <4 *Campylobacter* spp./plate, then the result was reported as containing *Campylobacter* but at a level of <40 c.f.u./g of sample. A single colony of *Campylobacter* spp. was fully identified to species level when available.

In this study, a carcase sample was declared enumeration positive if at least one confirmed colony of *Campylobacter* spp. was isolated.

Statistical methods

Data were analysed using Microsoft® Excel and Stata v. 10 (StataCorp, USA).

The McNemar test was used to assess differences between mCCDA and Preston agar and $P<0{\cdot}05$ was regarded as statistically significant. For measuring the agreement between *Campylobacter* detection by plating and enumeration, the kappa statistic (κ) was used. The interpretation used for Cohen's kappa statistic was: poor agreement $\kappa\leqslant 0{\cdot}20$, fair agreement $0{\cdot}21\leqslant\kappa\geqslant 0{\cdot}40$, moderate agreement $0{\cdot}41\leqslant\kappa\geqslant 0{\cdot}60$, substantial agreement $0{\cdot}61\leqslant\kappa\geqslant 0{\cdot}80$, and good agreement $\kappa\geqslant 0{\cdot}81$.

The outcome in the descriptive univariable analysis was a highly contaminated *Campylobacter* carcase (a neck and breast skin sample taken from one carcase within a specified slaughter batch with at least 1000 c.f.u./g). The baseline group included all carcases that were found to be negative for *Campylobacter* or carcases with a count of <1000 c.f.u./g. Continuous variables, apart from age, were recoded as categorical variables according to approximate centile distribution. Univariable logistic regression models were used to screen for potential risk factors. All exposures with a P value $<0{\cdot}25$ (χ^2 test or Wald test statistic) were assessed for inclusion in the multivariable logistic regression model. Variables were entered into the model in a forward stepwise fashion and only variables with a P value $<0{\cdot}05$ (likelihood-ratio χ^2 test or P_{Wald}) were retained. A backwards stepwise exclusion of non-significant exposures from the univariable analysis was then performed and any exposure with a P value $>0{\cdot}05$ (likelihood-ratio χ^2 test or P_{Wald}) was removed to obtain the simplest model. The rejected exposure variables were then individually re-introduced to assess for confounding and change in model fit (assessed by the Hosmer–Lemeshow goodness-of-fit test). A re-introduced variable was kept in the model if it changed any of the estimated odds ratios by more than 20% (confounding). Ordinal categorical variables were included as a linear or factor variable based on the P value from the likelihood-ratio χ^2 test. The variables in the final model were tested for biologically plausible interactions. The variable 'abattoir' was included using the cluster command to adjust for multilevel dependencies in the data between slaughter batches processed by the same abattoir.

The final model was based on 336 slaughter batches. Of the 400 eligible slaughter batches tested for *Campylobacter*, 28 were excluded as previous partial depopulation status was not known. Seventeen slaughter batches were excluded from the logistic regression model when region of farm (country) was included and a further 19 slaughter batches were dropped from the model when poultry company (six batches), type of flock (four batches), type of chilling used (four batches), number of birds on the holding (two batches), mortality at 14 days (two batches) and time from farm to slaughter (one batch) were included.

RESULTS

Three-quarters of the 400 eligible slaughter batches were sampled at abattoirs located in England (301, 75%), followed by Northern Ireland (44, 11%), Wales (34, 9%) and Scotland (21, 5%). The numbers of batches sampled by region were found to be proportionate to their annual broiler slaughter throughput; 74% (538 million broilers) in England, 13% (96 million) in Northern Ireland, 6% (43 million) in Wales and 6% (43 million) in Scotland. The number of slaughter batches sampled at each abattoir varied from one to 50 slaughter batches.

The majority of the slaughter batches (94·0%) originated from conventionally produced broilers with the remainder coming from free-range (standard) and organic farms [16 (4·0%) and 8 (2·0%), respectively]. This is similar to the structure of the broiler production system in England [21].

Prevalence of *Campylobacter*

Campylobacter spp. were detected in the caecal samples from 303 (75·8%) of the 400 eligible slaughter batches. Three-quarters (226, 74·6%) were identified as *C. jejuni* and 77 (25·4%) were *C. coli*. By comparison 349 (87·3%) of the carcases were confirmed as *Campylobacter* positive by one of the two methods (parallel detection by mCCDA and/or Preston; and/or enumeration). mCCDA was more effective at detecting *Campylobacter* (336, 84·0%) than Preston agar (290, 72·5%; $P<0{\cdot}0001$).

Campylobacter spp. were detected (by mCCDA and/or Preston) on 342 (85·5%) of the 400 carcases. Of the 342 positive carcases, *C. jejuni* was detected on 239 (69·9%) carcases and *C. coli* on 82 (24·0%) carcases. A further 21 (6·1%) carcases yielded *C. jejuni* and *C. coli* (Table 1).

The enumeration method detected *Campylobacter* on 68·3% (273) of the carcases; 83·5% (228) were identified as *C. jejuni* and 16·5% (45) as *C. coli* (Table 1). A moderate agreement between the parallel detection method (by mCCDA/Preston) and the

Table 1. Campylobacter *on broiler carcases by detection and enumeration methods*

	Number (%) positive by detection/ enumeration method				
	mCCDA	Preston	Combined detection*	Enumeration	Combined method†
C. jejuni	245 (72·9%)	211 (72·8%)	239 (69·9%)	228 (83·5%)	232 (66·5%)
C. coli	91 (27·1%)	79 (27·2%)	82 (24·0%)	45 (16·5%)	44 (12·6%)
Mixed			21 (6·1%)		73 (20·9%)
Total (%) positive	336 (84·0%)	290 (72·5%)	342 (85·5%)	273 (68·3%)	349 (87·3%)

* Detection by mCCDA and/or Preston.
† Detection (by mCCDA/Preston) and/or enumeration.

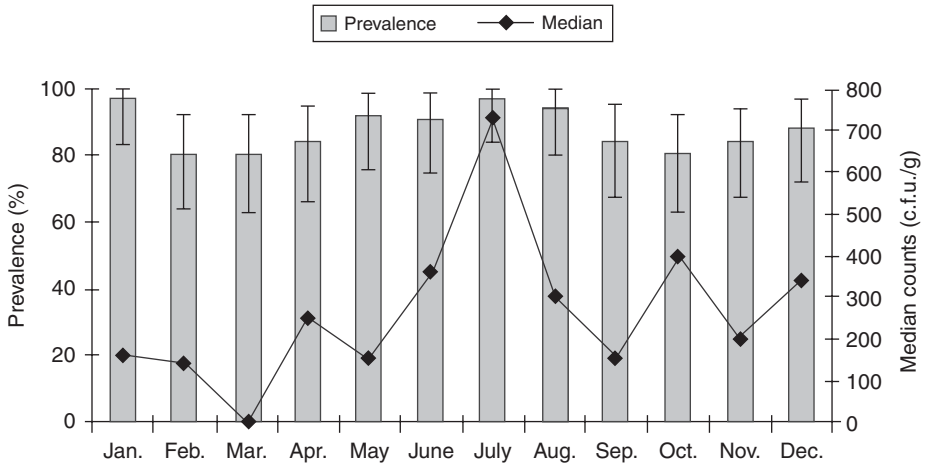

Fig. 1. Prevalence of carcase contamination and median counts of *Campylobacter* on carcases by month.

enumeration method was observed ($\kappa = 0{\cdot}44$). The prevalence of *Campylobacter* contamination of carcases varied from 80% [95% confidence interval (CI) 63·1–91·6] in March and October, to 97·0% (95% CI 84·2–99·9) in July (Fig. 1).

Quantification of *Campylobacter* on broiler carcases

The lowest limit for detection of *Campylobacter* was 5 c.f.u./g, where four carcases were found contaminated with *C. jejuni*, in contrast the highest count on neck and breast skin samples was 110 000 c.f.u./g. The level of contamination of *Campylobacter* on the 273 carcases is given in Table 2 and Figure 2. The level of *Campylobacter* contamination on carcases varied by *Campylobacter* caecal status. For batches recorded as *Campylobacter* negative by caecal sampling, the median *Campylobacter* count was 0 c.f.u./g (range 0–62 000) compared to 430 c.f.u./g (range 0–110 000) for *Campylobacter* (caecal)-positive batches. In conventional flocks, the median *Campylobacter* load was 235 c.f.u./g (range 0–110 000) compared to 125 c.f.u./g (range 0–3100) and 175 c.f.u./g (range 0–6200) for free-range and organic flocks, respectively. During the year the median *Campylobacter* count by month ranged from 0 to 730 c.f.u./g (Fig. 1).

Of the 400 batches enumerated, 109 (27·3%) batches were found to have counts $\geqslant 1000$ c.f.u./g. A seasonal variation in the proportion of carcases that were highly contaminated with *Campylobacter* ($\geqslant 1000$ c.f.u./g) was observed, from 19·2% (95% CI 12·0–28·3) in spring (March, April, May) to 39·8% (95% CI 30·0–50·2) in summer (June, July, August) (Fig. 3).

Comparison of the detection of *Campylobacter* in caeca and on broiler carcases

Overall, 296 slaughter batches were positive for *Campylobacter* spp. by both caecal and carcase sampling and both methods of detection (enrichment/plating and enumeration). A further 44 were negative by both sampling methods (Table 3).

Table 2. *Level of contamination on carcases:* Campylobacter *load by species*

		Campylobacter enumeration (c.f.u./g)						
Species detected	Total	<5	<10	10–39	40–99	100–999	1000–10 000	>10 000
C. coli	45			1	2	17	19	6
C. jejuni	228		4	14	18	108	71	13
No. of batches (%)	400		4 (1·0%)	15 (3·8%)	20 (5·0%)	125 (31·3%)	90 (22·5%)	19 (4·8%)

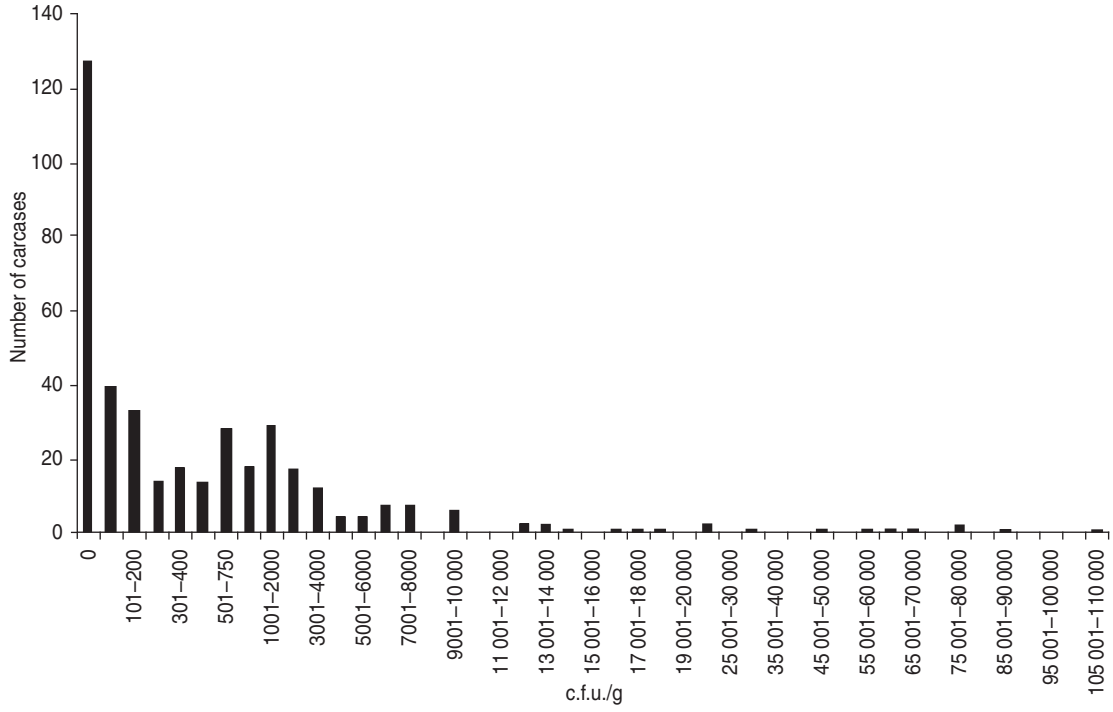

Fig. 2. Level of *Campylobacter* contamination (number of carcases by c.f.u./g).

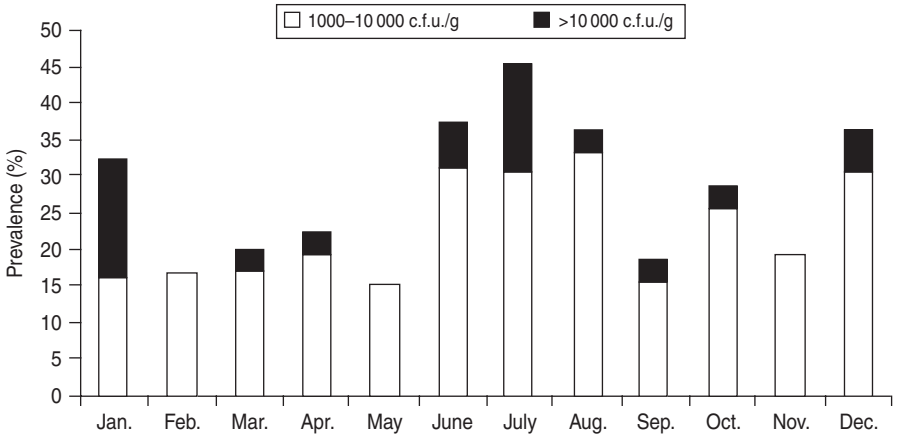

Fig. 3. Prevalence of carcase *Campylobacter* contamination ⩾1000 c.f.u./g by month.

Campylobacter spp. were cultured from the caeca of seven (13·7%) slaughter batches when *Campylobacter* was not detected on the carcase (by enrichment/plating and/or enumeration) from the same slaughter batch but in most (86·3%) batches the caeca were negative if the carcase was negative. Only

Table 3. *Detection of* Campylobacter *in slaughter batches, in caeca and broiler carcases* (*by detection and/or enumeration*)

	Carcase		
Caeca	Negative	Positive	Total
Negative	44	53	97
Positive	7	296	303
Total	51	349	400

45% of carcases were uncontaminated post-chilling from caeca-negative slaughter batches. In 53 (54·6%) slaughter batches, *Campylobacter* spp. were detected on the carcase but not in the corresponding caecal sample.

Identification of risk factors for highly *Campylobacter*-contaminated carcases

Of the 23 variables tested, 14 were associated with highly *Campylobacter*-contaminated carcases (Table 4). Company, season, previous partial depopulation, mortality (% of birds) recorded at 14 days and location of farm (country) were included in the multivariable model as independent risk factors. Age of the birds was included in the model as an *a priori* confounder. The type of crate used to transport the birds, type of chilling used at the abattoir, number of broilers on the holding, production type, slaughter schedule and length of time from farm to slaughter were all included in the final model as potential confounders (when each variable was individually included in the model the company-specific odds ratio for *Campylobacter* contamination changed by more than 20%). The model outputs for the exposures significantly associated with heavy *Campylobacter* carcase contamination are shown in Table 5.

DISCUSSION

This paper focuses on the UK results from the EU baseline survey on *Campylobacter* and an additional risk factor analysis of the most highly *Campylobacter*-contaminated carcases. The results of the survey for all of the Member States have been reported elsewhere [22].

The prevalence of *Campylobacter* on broiler carcases in the UK was 87·3%, this compares with 4·9–100% from the baseline surveys in the other European countries and is similar to the results from France [23]. The prevalence of *Campylobacter* in broiler batches and on broiler carcases is comparable to previous UK studies [14, 16]. However, direct comparison of results between studies is difficult and should be made with caution as both the sampling scheme and isolation method may vary between studies. Indeed, the *Campylobacter* prevalence observed in our study is much higher than other UK studies which excluded flocks that had been previously partially depopulated [12, 13]. However, we believe the high coverage of this UK-wide survey (88% of the total annual kill), combined with the randomized sampling approach provides a representative estimate of the *Campylobacter* prevalence in UK broiler flocks.

The prevalence of *Campylobacter* on carcases was higher compared to the prevalence obtained by caecal sampling (87·3% *vs.* 75·5%); 53 (13·2%) slaughter batches were classified as negative by caecal sampling but were positive by carcase sampling. The observed difference in prevalence is indicative of cross-contamination of the carcases with *Campylobacter* spp. during the slaughter process which concurs with other studies [18, 23–26].

The sampling of one carcase per slaughter batch may underestimate the prevalence of *Campylobacter* on broiler batches. Seven (1·8%) slaughter batches were identified as negative via carcase sampling; however, *Campylobacter* was detected in caeca from birds in the same batch. It is not known whether the numbers of *Campylobacter* in the caecal samples from these seven batches were low and potentially indicative of recent flock colonization. A positive correlation between the number of campylobacters in caeca and the number found on carcases has been observed [27]. However, most batches were detected as *Campylobacter*-contaminated by both caeca and carcase sampling.

The *Campylobacter* carcase prevalence and enumeration results from this study have been used, in part, to agree a voluntary industry target with the UK government to reduce the proportion of birds in the most contaminated group (>1000 c.f.u./g). The target will be measured at UK slaughterhouses and has been set from a baseline of 27% in 2008, based on this survey, to achieve a reduction to 10% by 2015. In light of this target, we undertook a risk analysis to examine any factors associated with the most highly contaminated carcases. The aim of the risk-factor analysis was to generate advice to industry which could help to reduce the proportion of highly

Table 4. *Univariable analysis: association between exposure variables and highly* Campylobacter-*contaminated carcases* (*⩾1000 c.f.u./g*)

Variable	Category (no. of carcases)	Highly *Campylobacter* contaminated carcases* (%)	Unadjusted OR	95% CI	*P* value
Poultry company					0·007
	12 (91)	14·3	1·00		
	1 (50)	22·0	1·69	0·69–4·12	
	2 (21)	14·3	1·88	0·59–6·00	
	3 (40)	25·0	2·00	0·79–5·05	
	4 (8)	50·0	6·00	1·33–27·0	
	5 (52)	46·2	5·14	2·31–11·5	
	6 (16)	35·7	3·60	1·12–11·6	
	7 (3)	33·3	3·00	0·25–35·5	
	8 (5)	80·0	24·0	2·48–231·9	
	9 (5)	20·0	1·50	0·16–14·5	
	10 (5)	20·0	1·50	0·16–14·5	
	11 (15)	26·7	2·19	0·60–7·90	
	13 (10)	20·0	1·50	0·29–7·87	
	14 (1)	100·0			
	15 (2)	0·0			
	16 (2)	0·0			
	17 (74)	27·0	2·54	1·18–5·48	
Abattoir					<0·0001
Region (location of abattoir)					0·052
Bird age (days)					0·011
	<36 (95)	17·9	1·00		
	36–38 (61)	21·3	0·82	0·38–1·76	
	39–41 (98)	29·6	1·58	0·70–3·56	
	42–48 (96)	37·5	0·85	0·27–2·70	
	⩾49 (50)	20·0	0·13	0·16–1·13	
Month of slaughter					0·072
	January (31)	32·3	1·00	.	
	February (36)	16·7	0·42	0·13–1·33	
	March (35)	17·1	0·53	0·17–1·61	
	April (31)	19·4	0·61	0·20–1·90	
	May (33)	15·2	0·38	0·11–1·26	
	June (32)	34·4	1·26	0·45–3·56	
	July (33)	45·5	1·75	0·63–4·84	
	August (33)	36·4	1·20	0·43–3·38	
	September (32)	18·8	0·48	0·15–1·55	
	October (35)	25·7	0·84	0·29–2·40	
	November (36)	19·4	0·51	0·17–1·55	
	December (33)	36·4	1·20	0·43–3·38	
Season of slaughter					0·006
	Spring (99)	17·1	1·00		
	Summer (98)	38·8	2·78	1·46–5·30	
	Autumn (103)	21·4	1·21	0·61–2·39	
	Winter (100)	28·0	1·64	0·84–3·18	
Production type					0·394
	Conventional (376)	26·9	1·00		
	Free range (16)	12·5	0·19	0·03–1·51	
	Organic (8)	25·0			
First batch slaughtered from flock					<0·001
	First batch slaughtered (138)	12·3	1·00		
	Previously partly depopulated (234)	34·2	3·99	2·25–7·07	
No. of broilers in shed/enclosure					0·569
	<20 000 (108)	25·0	1·00		
	20 000–29 999 (113)	22·1	1·00	0·53–1·87	
	⩾30 000 (166)	30·1	1·32	0·75–2·31	

Table 4 (*cont.*)

Variable	Category (no. of carcases)	Highly *Campylobacter* contaminated carcases* (%)	Unadjusted OR	95% CI	*P* value
					0·202
No. of broilers on holding					
	<100 000 (133)	21·8	1·00		
	100 000–199 999 (137)	28·5	1·61	0·91–2·85	
	⩾200 000 (115)	28·7	1·81	1·01–3·27	
Flock mortality at 14 days (% birds)					0·492
	<1·25 (148)	24·3	1·00		
	1·25–1·749 (129)	29·5	1·35	0·80–2·28	
	⩾1·75 (120)	25·0	1·00	0·60–1·82	
Flock mortality at 72 h before slaughter (% birds)					0·916
	<2 (90)	25·6	1·00		
	2·0–2·74 (110)	26·4	1·15	0·60–2·18	
	2·75–3·49 (69)	24·6	0·95	0·45–2·00	
	⩾3·50 (94)	28·7	1·25	0·64–2·42	
Recent mortality (% birds)†					0·614
	<1·00 (147)	27·2	1·00		
	1·00–1·49 (106)	23·6	0·81	0·45–1·45	
	⩾1·50 (108)	28·7	1·07	0·61–1·87	
Rejects at slaughter (% birds)					0·650
	<0·75 (140)	26·4	1·00		
	0·75–1·249 (109)	22·9	0·79	0·43–1·42	
	⩾1·25 (116)	27·6	1·05	0·60–1·83	
Type of crate used to transport birds					0·001
	Open floor (264)	22·7	1·00		
	Solid (84)	44·1	0·61	0·23–1·66	
	Open floor and solid (31)	12·9	2·51	1·50–4·21	
	Not known (21)	19·1	0·75	0·24–2·31	
Time in transit to abattoir (hours)					0·781
	<1 (84)	28·6	1·00		
	1·0–1·49 (89)	27·0	0·74	0·37–1·48	
	1·5–2·49 (121)	29·8	0·98	0·53–1·83	
	⩾2·5 (105)	23·8	0·77	0·40–1·48	
Time in transit and lairage (hours)					0·930
	<2·50 (96)	26·0	1·00		
	2·50–3·49 (105)	29·5	1·16	0·61–2·18	
	3·5–4·74 (93)	25·8	0·94	0·48–1·85	
	⩾4·75 (105)	27·6	1·13	0·60–2·12	
Slaughter schedule					0·722
	Before 06:00 hours (44)	25·0	1·00		
	After 06:00 hours (356)	26·4	1·14	0·55–2·34	
Type of chilling used					0·041
	Air (367)	29·2	1·00		
	Immersion (3)	0·0			
	Air and immersion (1)	0·0			
	Air and spray (29)	6·9	0·18	0·04–0·77	
Salmonella status					0·182
	Negative (386)	26·7	1·00		
	Positive (14)	42·9	2·06	0·70–6·08	
Country (farm location)					0·124
Region – NUTS1 (farm location)					0·227
Region – NUTS2 (farm location)					0·015

OR, Odds ratio; CI, confidence interval.

* Carcases with a count ⩾1000 c.f.u./g.

† Mortality at 72 h before slaughter minus mortality at 14 days.

Table 5. *Multivariable analysis: risk factors** *associated with highly* Campylobacter-*contaminated carcases* (*⩾1000 c.f.u./g*)†

Exposures	OR	95% CI	*P* value
Previous partial depopulation‡			
Yes	3·37	1·38–8·20	0·007
Season§			
Summer	3·50	1·54–7·93	0·003
Mortality at 14 days¶			
1·25% to <1·75%	2·54	1·10–5·88	0·030
Company‖			
Company 4	4·49	1·55–13·0	0·006
Company 6	5·83	1·74–19·5	0·004
Company 8	110·6	20·2–604·3	<0·001
Company 11	24·3	3·09–191·9	0·002

OR, Odds ratio; CI, confidence interval.

* Region (country) of farm was included in the multivariable model as an independent risk factor (data not shown).

† The following exposures were also included in the multivariable model:

- Age of the birds was included as an *a priori* confounder.
- Type of crate used to transport the birds, type of chilling used, number of birds on the holding, type of flock, slaughter schedule, and time from farm to slaughter were included in the model as potential confounders (the company-specific odds ratio for *Campylobacter* contamination changed by more than 20% when each variable was added to the model).

‡ Baseline: First batch slaughtered from flock.

§ Baseline: Spring (March, April, May).

¶ Baseline: Mortality <1·25%.

‖ Baseline: Company 12.

Campylobacter-contaminated carcases and thus help the UK to meet the new target.

A significantly increased risk of highly contaminated carcases was observed in the summer months (July, August, September; OR 3·50), this is consistent with other European studies which have shown a seasonal summer peak in *Campylobacter* colonization or carcase contamination [28–32]. The observed increase in risk may be explained by changes in environmental reservoirs and the frequency in which flocks are exposed to the organism, in addition to changes in climatic factors such as temperature and precipitation. The role of flies in the epidemiology of *Campylobacter* has been suggested by several authors [33, 34], a peak in the *Campylobacter* carriage rate of flies during July to August has been observed which may represent a risk in colonization of broiler flocks [35]. A recent study which examined the incidence of *Campylobacter* in broilers and people in six Northern European countries (Denmark, Finland, Iceland, Norway, Sweden, The Netherlands) found that temperature-related factors were probably in part responsible for the seasonality in incidence [30]. Rigorous or enhanced biosecurity measures have been shown to reduce *Campylobacter* colonization in broiler flocks [36]. In a recent study in Sweden, a good general tidiness score and an increase in the number of changes of footwear were both associated with a decreased risk of colonization [37]. Enhanced biosecurity should be advocated particularly during the summer months; however, such measures may not be effective in reducing the high levels of *Campylobacter* contamination. Given the complexity of *Campylobacter* epidemiology in broilers, and the observed increased risk of highly contaminated carcases during the summer months, further research to examine the factors associated with seasonality should be undertaken.

The risk for high *Campylobacter* contamination increased if the flock had been previously partially depopulated (OR 3·37), this is consistent with other studies [28, 37, 38]. This association was independent of the age of birds. In this study, previously partially depopulated flocks included flocks that had been partially depopulated the previous day or up to 20 days prior to our sampled batch (mean and median of 7 days). We were unable to examine any potential risks associated with the length of time between previous partial depopulation and slaughter as this information was missing from a large number of batches; however, this length of time may be relevant in attempting to minimize the proportion of birds that are most highly contaminated and warrants further investigation.

An increase in the mortality of birds in the first 2 weeks of life was shown to increase the risk of heavily contaminated carcases (OR 2·54) although a linear trend was not observed [a high mortality of ⩾1·75% was not associated with heavily contaminated carcases (OR 1·55, 95% CI 0·71–3·37)]. The increase in risk is consistent with a larger study (of which this study was a part), which found an association between higher recent flock mortality and an increased risk of *Campylobacter* infection in broiler flocks at slaughter [28]. Furthermore, a higher level of rejection due to infection and digital dermatitis were both found to be associated with *Campylobacter*-positive slaughter batches [39]. Although *Campylobacter* is generally

regarded as a commensal in broilers, these findings may suggest that *Campylobacter* is more common in flocks compromised by poor health. Conversely, the mortality rate may be a proxy marker for farm management practices or biosecurity on the farm. The absence of a linear trend may reflect farm management practices in that when higher mortality rates are recorded on the farm, the situation is managed by the use of antibiotics, for example, and the actions in response to the higher mortality rate impacts upon *Campylobacter* colonization and/or contamination.

Poultry company was identified as an independent risk factor for highly contaminated carcases. Ten companies were shown to have an increased risk of high levels of carcase contamination compared to the baseline group and of these, four companies had a significantly increased risk. The number of slaughter batches sampled by these four companies ranged from five to 16 batches (Table 4). Differences in *Campylobacter* colonization and contamination of carcases by producer/abattoir have been reported elsewhere [26]. However, it should be noted that due to the large number of companies involved in the study and the small sample size for several companies, the confidence intervals for the odds ratios are very wide and the upper limits should be treated with caution. However, the results are indicative of company-specific risk factors or possible recirculation of strains within a company and as such warrant further investigation.

Farm location was identified as a significant factor associated with highly contaminated carcases (odds ratio for regional data not shown). Although the multivariable model included company as an independent risk factor, and therefore the outputs would have been adjusted for company-related effects, it is possible that abattoir-specific or farm-related factors, not measured by our study, confound this finding. Alternatively, this exposure variable could be a proxy for geographical or climate factors. An increased risk of *Campylobacter* colonization in relation to geographical areas has been observed in previous studies [40] including the EU baseline survey [22]. Furthermore, an association between *Campylobacter* prevalence and climatic factors has recently been reported in Great Britain (GB) [32], in this study *Campylobacter*-positive flocks were more likely to be reared in northern GB. This finding should be further explored.

An increased risk of *Campylobacter* prevalence with increasing age has been well documented [13, 28, 41]. In our study age was not significantly associated with high levels of carcase contamination in the multivariable analysis.

Crates used to transport broilers to the abattoir can be contaminated with *Campylobacter* and as such pose a potential risk for the transmission or contamination of birds [42]. Research has shown that isolates can survive on the crates post-sanitization [43, 44]. An increased risk of heavy *Campylobacter* contamination of carcases was observed in birds transported in solid and open crates (OR 3·56 95% CI 0·99–12·8), compared to open crates alone; however, this increase in risk was of borderline significance.

The design and implementation of successful control programmes at the farm and abattoir level require a better understanding of the epidemiology of *Campylobacter* in broiler flocks. A review of risk assessments on *Campylobacter* in broiler meat found that the most effective intervention measures should focus on reducing the numbers of *Campylobacter* rather than prevalence alone [45]. In the UK, a voluntary industry target to reduce the most contaminated slaughter batches has been agreed with government and this study is the first to describe the risks associated with such contamination.

CONCLUSION

Slaughter during the summer months, flocks that have been previously partially depopulated and an increased mortality rate were found to have an increased risk of high *Campylobacter*-contamination levels on carcases post-chill (counts of $\geqslant$1000 c.f.u./g). Enhanced biosecurity and efforts to minimize *Campylobacter* contamination should be paramount during the summer months and when flocks are thinned, while further efforts to understand the complex epidemiology of *Campylobacter* infection are undertaken. The risk of heavily contaminated carcases varied by poultry company, and we recommend examining the practices and procedures in place within companies to try to identify areas for reducing carcase contamination. A regional effect on the risk of high *Campylobacter* contamination was also observed hence the potential role of climatic or abattoir-/farm-level factors should be further investigated.

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DECLARATION OF INTEREST

None.

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