

Influence of Season and Geography on *Campylobacter jejuni* and *C. coli* Subtypes in Housed Broiler Flocks Reared in Great Britain[∇]

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Geographical and seasonal variation in the incidence and prevalence of *Campylobacter jejuni* and *C. coli* in housed broiler flocks reared in Great Britain in 2004 to 2006 was investigated in this study. Ceca (30) from 797 flocks, not subject to prior partial depopulation and reared on 211 farms, were examined individually for the presence of *Campylobacter* spp. The best-fitting climatic factors explained approximately 46% of the prevalence of *Campylobacter*-colonized flocks at slaughter and consisted of a combination of temperature at slaughter, number of sunshine hours in placement month, and millimeters of rainfall in placement month. Positive flocks were more likely to be slaughtered between June and November than during the rest of the year and to be reared in northern Great Britain than in central or southern Great Britain. *C. jejuni* was identified in approximately 90% of flocks, and *C. coli* was present in 10% of flocks. The most common clonal complexes identified in 226 isolates typed by multilocus sequence typing (MLST) were ST-45, ST-21, ST-574, ST-443, and ST-828. Flocks slaughtered at the same time were more likely to have similar complexes, and ST-45 had a seasonal pattern, with the highest prevalence in June, and was also more likely to be present in flocks reared in northern Great Britain.

Campylobacter spp., especially *Campylobacter jejuni* and *C. coli*, are the main cause of human bacterial gastroenteritis in the developed world (<http://www.who.int/mediacentre/factsheets/fs255/en/>). Chicken meat is frequently contaminated with *Campylobacter* (19), and it is a commonly held view that reducing the number of flocks infected with this organism would reduce the number of human *Campylobacter* cases. A better understanding of the epidemiology of *Campylobacter* in broiler flocks is required in order to design successful control programs at the farm level.

In some European countries, flock colonization of chickens with *Campylobacter* has a clear seasonal pattern, with highest rates seen in the summer or autumn (20). Studies in the United Kingdom in the early 1990s suggested that there was no increase in the proportion of flocks colonized with *Campylobacter* in warmer months (21, 28). However, we recently found

that housed flocks were more likely to be *Campylobacter* positive in summer (46) in a geographical subset of the data in this study, and evidence of seasonality was also found in 401 batches reared in the United Kingdom in 2008 (20). The reasons for the seasonal variation are not fully understood but are likely to involve the frequency and nature of exposure of the flocks to *Campylobacter* spp. There is further evidence that climatic factors such as temperature correlate with both broiler flock and human infections (36, 43, 52). Temperature could also affect the environmental sources of *Campylobacter* spp. to which broiler chickens may become exposed. A better understanding of the roles of season and climatic factors and their relative impacts on broiler flock colonization with *Campylobacter* will be useful for policy makers and broiler companies who are formulating control programs to reduce flock infection with this important zoonotic pathogen.

Typing of isolates from foods and clinical cases has provided evidence that many strains isolated from chickens share attributes with those from human cases (13, 25, 37). Multilocus sequence typing (MLST) has been used to assess the relative importance of sources, reservoirs, and transmission routes for human *Campylobacter* infection in the United Kingdom (48, 56). The population of *Campylobacter* isolates from human cases in England has been well analyzed by studies of isolates from >2,900 cases from three areas spanning the years 2000 to 2006 (14, 56). However, data sets for strains from chicken flocks at slaughter were smaller and may have been less representative. For example, while 307 chicken isolates were cited

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by Wilson et al. (56), only 248 of those were from the United Kingdom, and of those, 177 isolates were from one study (9) and comprised only six broiler flocks.

This study aimed to examine geographical, seasonal, and climatic factors associated with *Campylobacter* prevalence in broiler flocks and to ascertain the distribution of sequence types (STs) of isolates from flocks reared mainly in England from 2003 to 2006. The establishment of larger and better-sampled MLST data sets has recently been highlighted as an important tool to improve public health interventions (49). We also wanted to investigate whether there was any clustering of isolates, as has been observed for some human strains (14) and strains isolated from chickens reared in New Zealand (41).

MATERIALS AND METHODS

The flocks in this study were reared by three integrated poultry companies in Great Britain between December 2003 and March 2006. All birds were reared indoors, and samples were collected from flocks at first partial or full depopulation only.

Campylobacter isolation. For each slaughter group, up to 30 intact pairs of ceca were collected from the birds and examined for *Campylobacter* as described by Bull et al. (8). Briefly, this involved plating cecal content from each individual cecum onto one modified charcoal-cefoperazone-deoxycholate agar (mCCDA) (Oxoid Ltd. CM739 agar with SR155 supplement) plate. Following incubation under standard microaerobic conditions, presumptive colonies were confirmed as *Campylobacter* spp. by use of standard tests (8).

Identification of *Campylobacter* species. *Campylobacter* isolates were recovered from frozen beads on Columbia blood agar (CBA). A DNA template was prepared by resuspending cells from a single colony in distilled H₂O (dH₂O) (500 μ l) and then heating them at 100°C for 10 min. A PCR was set up using three primers designed to simultaneously identify the *hipO* gene of *C. jejuni*, the *glyA* gene of *C. coli*, and the 23S rRNA gene of *Campylobacter* spp. (54). Each PCR mixture contained 25 μ l HotStarTaq DNA polymerase (Qiagen), 4 μ l of magnesium chloride (50 mM), 4 μ l of primer mix (resulting in 0.07, 0.1, and 0.01 μ M [each] concentrations of the *C. jejuni*, *C. coli*, and 23S rRNA forward and reverse primers), 1 μ l of template DNA, and 16 μ l of nuclease-free water. DNA amplification was carried out in an engine thermocycler (MJ Research, MA) under conditions specified by Wang et al. (54), with the following modification: an initial denaturation step of 95°C for 15 min was used. The PCR products were analyzed by gel electrophoresis through 2% (wt/vol) agarose containing 1 μ g ml⁻¹ ethidium bromide in 1 \times Tris-acetate-EDA (TAE) buffer. The DNA bands were visualized using an ultraviolet transilluminator (BioDoc-It imaging system; UPV).

***flaA* SVR types and MLST.** Isolates were further characterized by sequencing the short variable region (SVR) of the *flaA* gene. Crude cell lysates were prepared as described above, although occasionally a DNA extraction was carried out by resuspending a substantial loopful of colony mass in 3 ml 1 M Tris with 5 μ l ml⁻¹ lysozyme (Fisher) and adding 2 ml EDTA (0.5 M at pH 8.0). Cells were then incubated at 37°C for 1 h, followed by a short incubation with 0.5 ml of 10% SDS at 42°C. Once cell lysis had occurred, 2.5 ml phenol-chloroform-isoamyl alcohol (25:24:1 mixture, pH 8) was added, and the tube was shaken and then centrifuged at 10,000 \times g for 20 min at 11°C. The uppermost layer was taken off and added to a new centrifuge tube, half its volume of isopropanol was added, and the mixture was left on ice for 1 h for the DNA to precipitate. Samples were centrifuged at 10,000 \times g to pellet the DNA, the supernatant was discarded, and the pellet was washed with ethanol. The pellet was left to dry to remove all traces of ethanol and then resuspended in 100 μ l dH₂O and stored at -20°C until required.

The *flaA* gene was amplified using forward and reverse primers published by Nachamkin et al. (42) and Meinersmann et al. (38). The reaction mix and PCR conditions specified by Nachamkin et al. (42) were used. Amplification was confirmed by running 5 μ l of the PCR product in a 1% (wt/vol) agarose gel, and the rest of the product was cleaned up by precipitation with 20% (wt/vol) polyethylene glycol, 2.5 M NaCl (17), or by using a QIAquick PCR purification kit (Qiagen). Nucleotide sequences were determined on both DNA strands, using the corresponding forward and reverse primers (0.67 μ M) and BigDye Ready Reaction mix (Applied Biosystems). Unincorporated dye terminators were removed by precipitation with 100% ethanol and 3 M sodium acetate, followed by centrifugation at 2,750 \times g. The reaction products were separated

and detected using an ABI Prism 3770, 377, or 3730 automated DNA sequencer (PE Biosystems, Warrington, United Kingdom). Sequences were assembled from the resultant chromatograms with the STADEN suite of computer programs and assigned an allele number from the PubMLST *Campylobacter* FlaA database (<http://pubmlst.org/campylobacter/>).

MLST was performed using seven housekeeping genes, i.e., *aspA* (aspartase A), *gluA* (glutamine synthase), *gluA* (citrate synthase), *glyA* (serine hydroxymethyltransferase), *pgm* (phosphoglucosmutase), *tkt* (transketolase), and *uncA* (ATP synthase alpha subunit), with primers described by Dingle et al. (12) and Miller et al. (40) and modified reaction and amplification conditions. Separate 25- μ l PCR mixtures were set up for each target gene, using 12.5 μ l PCR master mix (Promega), 0.5 μ l each of forward and reverse primers (10 μ M; final primer concentration, 0.25 μ M), 6.5 μ l nuclease-free water, and 5 μ l template DNA. DNA amplification was carried out in a thermocycler under the following conditions: 95°C for 2 min, 35 cycles of amplification (94°C for 20 s, 50°C for 20 s, and 72°C for 1 min), and a final extension step of 72°C for 5 min. Amplification of the product was confirmed, and the product was cleaned up and sequenced as described above. Alleles, STs, and clonal complexes (CC) were assigned using the *C. jejuni* and *C. coli* PubMLST database (<http://pubmlst.org/campylobacter/>; accessed 20 June 2009), with new allele combinations submitted and STs designated as appropriate (the latter can be extracted from the PubMLST database by querying sender SHarris).

Farm, climatic, and geographic data. Information on the number of commercial premises rearing broilers indoors was obtained from the GB Poultry Register maintained by the RADAR Team at the Department for the Environment, Food and Rural Affairs (Defra) (www.defra.gov.uk; accessed 11 May 2006). A map of flock density was created from the grid references for the postcode district centroids (obtained from Graticule) for each farm, using Arcmap 9.1 software (ESRI, Redlands, California). The grid references were also sent to the Climate Production Department of the UK Met Office, who assigned them to the standard weather districts used by the organization: East Anglia, England E and NE, England NW and Wales N, England SE and Central, England SW and Wales S, Midlands, and Scotland W. The mean temperature (°C), total rainfall (mm), and hours of sunshine (h) were obtained from the Met Office website for each flock at time of placement and time of slaughter according to the weather district in which the farm was located (e.g., see <http://www.metoffice.gov.uk/climate/uk/2006/>). The mean value of each climate variable was calculated to estimate the climate during the rearing period.

To accommodate the data with regard to geographical location, the regions derived from the Met Office were combined into three larger areas: Northern GB (England E and NE, England NW and Wales N, and Scotland), Central GB (East Anglia and Midlands), and Southern GB (England SE, Central England, England SW, and Wales S).

Statistical analyses. Data checking and consistency checks were carried out prior to descriptive and statistical analyses. Any outliers were checked for transposition errors. The outcome variable was the *Campylobacter* status of the flock, where one or more positive ceca classified the batch as positive. The number of positive ceca within each batch among the total ceca tested was recorded as within-batch prevalence.

Geographical area and month and year of placement and slaughter were described and compared with the outcome variable "*Campylobacter* status of the flock" by using simple logistic regression models that accounted for clustering of flocks within farms. Wald's χ^2 statistics was used to assess the fit of each model as a measure of the ability of the climate variable to predict *Campylobacter* prevalence by month and year.

An analysis of the *Campylobacter* prevalence across the farms was undertaken with generalized estimating equations (GEE), as the residuals did not conform well to an appropriate error model. All climate variables were fitted into GEE models specified with robust standard errors, an exchangeable correlation matrix, a binary family, and logit link to allow for clustering of batches by farm. The multivariable climate model with the best fit (Wald's χ^2) was considered the best predictive combination, and observed and predicted values were compared in a scatter plot. R^2 statistics of the fit was interpreted as the proportion of the observed prevalence that was explained by climate. All analyses were performed using Stata 10 software (Stata, College Station, TX). Colinearity between the variables was examined, but none was found.

The variation in individual clonal complex types and *flaA* SVR types with farm and time of slaughter was investigated using a progressive model building strategy and a combination of generalized linear mixed-effect (GLMM) modeling and Monte Carlo permutation tests. In the first instance, the incidence/absence of individual clonal complexes in a flock was used as a response in a mixed-effect model with time expressed as two harmonic covariates and geographical location represented as National Grid Reference coordinates for easting and northing.

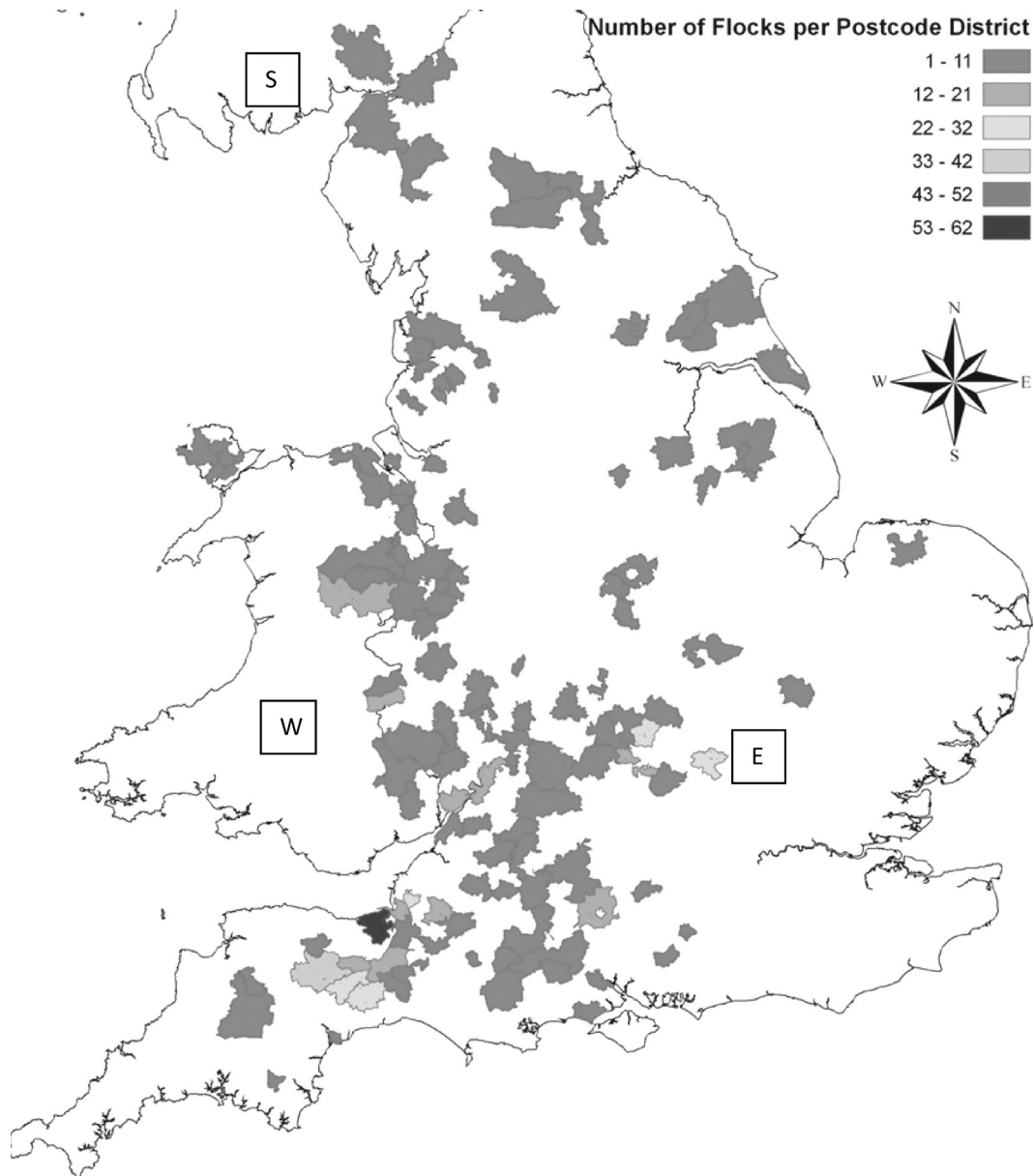


FIG. 1. Geographical distribution of sampled flocks. Approximate borders between Scotland (S), England (E), and Wales (W) are indicated.

We used the presence or absence of each clonal complex type in a flock as a response variable in logistic regression, following a penalized quasi-likelihood approach. In the second case, the extent to which the clonal complexes were found repeatedly on the same farm was assessed using Monte Carlo permutation approaches, whereby we compared the observed incidences of clonal complexes with the distributions obtained by permuting the incidences of cases among farms at random.

RESULTS

Seven hundred ninety-seven flocks from 211 farms were tested during the course of the study, comprising 13% of premises rearing >50 housed broiler chickens in Great Britain. The flocks were reared on farms that were located through much of England and Wales (Fig. 1), although premises in Southeast England, East Anglia, and Scotland were underrepresented.

Prevalence in relation to temporal and climatic factors. *Campylobacter* spp. were isolated from 286 of the 797 flocks examined (35.9%). The prevalence was significantly higher in the northern part of Great Britain than in the southern and central parts ($P < 0.001$), and a significant reduction in flock prevalence during the study was also observed ($P < 0.001$). The mean prevalence in the 313 batches sampled in 2004 was 48%, compared to 31% of 366 batches in 2005. A seasonal pattern was also observed, and the prevalence of positive batches was significantly higher in July (54%; $P = 0.01$), August (55%; $P = 0.005$), and September (60%; $P < 0.001$) than during the rest of the year (range, 14 to 48%). The summer peak was slightly more pronounced in Southern GB than in the other regions (Fig. 2). In some positive batches, not all ceca

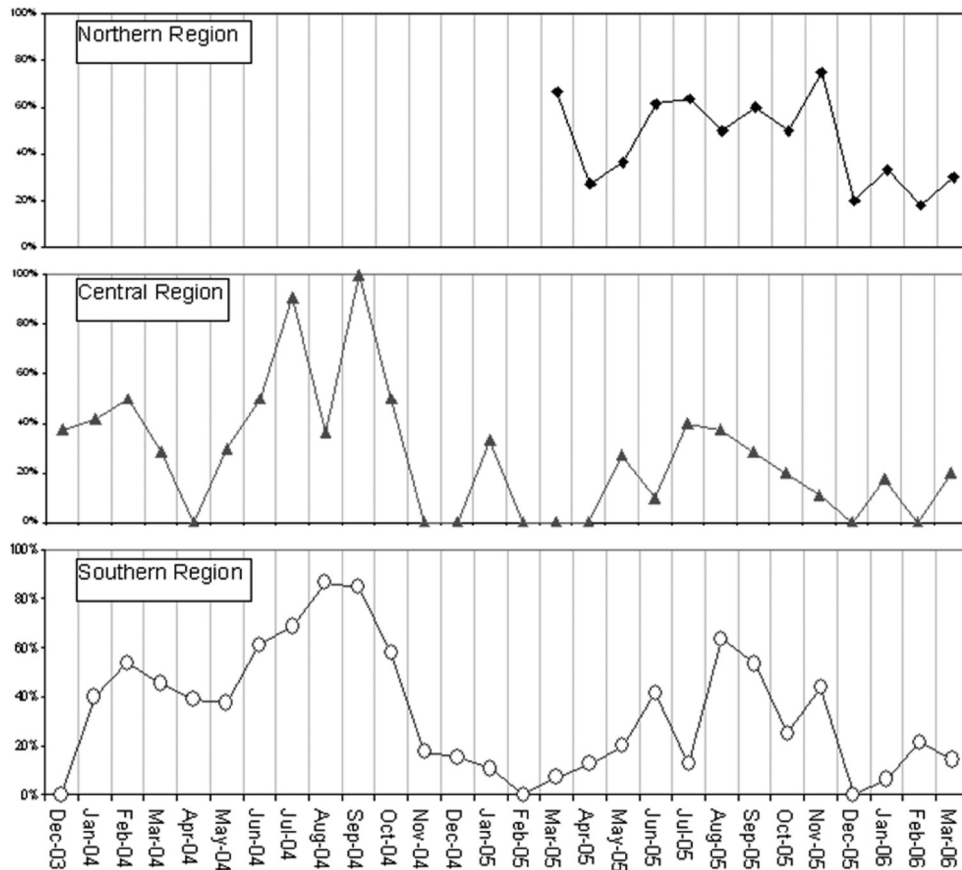


FIG. 2. Prevalence of *Campylobacter*-positive batches stratified by region from December 2003 to March 2006 (*n* = 797).

were *Campylobacter* positive, and 41% of such batches had a low within-batch prevalence (that is, 3 to 20% of the 30 ceca tested were *Campylobacter* positive), while 28% of flocks were colonized at a medium level (20.1 to 70%). The remaining 31% of flocks were highly colonized (>70 to 100% positive ceca) (Fig. 3).

There was very little variation in the climatic variables between the two full years of the study, 2004 and 2005. Generally, summer temperatures were about 1°C higher in central Great

Britain than in the southern regions and 1°C colder in the North. The North had fewer sunshine hours in summer than Central GB, whereas Southern GB had the most sunshine hours during the year. Amounts of rainfall, however, differed widely, with the highest rates in the autumn of 2004 in the South. The central region was consistently the driest, whereas both the South and the North had more seasonal variations in rainfall, with peaks in November and December 2005. The individual climate variables explained different proportions of the observed *Campylobacter* prevalence in broiler flocks and were dependent on when in the rearing cycle they were measured (Table 1). An overall trend showed that temperature was more important than sunshine and that rainfall and region explained less of the *Campylobacter* status than the other two variables. However, temperature explained only about half of the *Campylobacter* prevalence, and the high prevalence in the North despite the slightly lower temperature can likely be explained by other factors, such as husbandry and management. The multivariable climate model with the best fit was very different from the predictive values for the individual climate variables and consisted of temperature at time of slaughter, sunshine hours in the month of placement, and rainfall in the month of placement (Wald's $\chi^2 = 60.6$). All other combinations explained less of the *Campylobacter* status of the batches. Climate factors influenced the *Campylobacter* prevalence in batches of broilers, and temperature appeared to

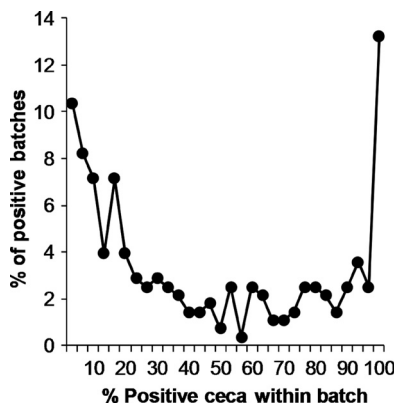


FIG. 3. Within-batch prevalence rates for *Campylobacter*-positive ceca in 283 positive broiler batches.

TABLE 1. Comparison of fits of univariable logistic regression models to identify climate variables that predict *Campylobacter* prevalence

Predictor	Model fit (Wald's χ^2)
Avg temp during rearing.....	51.0
Temp at slaughter.....	50.3
Sunshine hours during rearing.....	47.1
Temp at placement.....	46.7
Sunshine hours during placement.....	44.6
Sunshine hours at slaughter.....	36.5
Yr of slaughter.....	31.2
Rainfall at slaughter.....	5.6
Region.....	5.2
Rainfall during placement.....	3.4
Avg rainfall during rearing.....	0.0

be most important late in rearing, whereas sunshine and rainfall influenced prevalence in the beginning of the cycle, closer to placement. This combination of climate factors correlated well with the prevalence of *Campylobacter*-positive batches (Fig. 4) and explained about 46% of the observed prevalence.

***Campylobacter* sp. sequence types.** The typed isolates were proportionate to the numbers of positive flocks detected in each season and region of the sample. *C. jejuni* was identified in the majority of *Campylobacter*-positive flocks (209 flocks), but *C. coli* was also found (22 flocks). Identification to the species level of a second isolate from 147 flocks mostly identified the same species (143 flocks), while colonization with both *C. jejuni* and *C. coli* was detected in 4 flocks.

Isolates from 210 flocks were assigned to clonal complexes, while those from 16 flocks, including sequence types 531, 791, 877, 1080, 1237, 1376, 1492, 1495, 1597, 2216, 3472, and 3578, were not assigned to a clonal complex. Just over 50% of strains belonged to five clonal complexes (ST-45, ST-21, ST-574, ST-443, and ST-828) (Fig. 5), while each of the remaining complexes had fewer than 7% of isolates. All strains in clonal complexes ST-828 and ST-1150 were *C. coli*. The most prevalent STs were ST-45 (15 isolates), ST-574 (15 isolates), ST-51 (14 isolates), and ST-257 (8 isolates), and 81 STs (36% of isolates) appeared only once. The same clonal complexes appeared more often on the same farms than on different ones ($P = 0.047$). However, a temporal pattern was also present and indicated that flocks slaughtered at the same time were more likely to have similar complexes ($P = 0.002$). Isolates belonging to clonal complex ST-45 had a seasonal pattern, with the highest prevalence in June (showing a harmonic pattern with t values of -2.66 and -2.99 for the harmonic covariates at P values of 0.0079 and 0.0037 for cosine and sine covariates, respectively). These isolates were also significantly more likely to be present in flocks reared in the North than in the South ($t = 2.8$; $P = 0.0052$). None of the other common clonal complexes (we tested ST-21, ST-574, ST-443, and ST-828) showed any statistically significant seasonal or geographical patterns. The *flaA* locus provided additional resolution within STs, and in total, 67 different alleles were found. Five groups comprised 4 or more isolates where identical ST and *flaA* SVR types were present (Table 2). Of these, the *flaA* SVR105/ST-574 group was clustered in time compared to the others (0.27

month versus 0.42 to 0.63 month), but we did not detect any temporal patterns for other *flaA* SVR/ST types.

DISCUSSION

This study has demonstrated an association between the *Campylobacter* prevalence in housed chicken flocks and season and climate factors in Great Britain. The study has also established the ST distribution for a well-sampled set of isolates obtained from cecal contents at slaughter from chickens reared mainly in England. Temporal clusters were detected in only a small part of the observations in this study.

The study examined batches at first full or partial depopulation, representing a significant proportion of birds that go to slaughter. Partial depopulation has been reported as a risk factor for flocks being *Campylobacter* positive at slaughter (3, 5, 22), and our study purposely excluded this confounder to study other factors during rearing. The flock prevalence of *Campylobacter* found in this study (36%) is realistic compared to those reported in other European countries between 2004 and 2007 (in Norway, 3.1 to 5.2%; in Sweden, 12.6 to 14.2%; in Denmark, 26.8 to 29.9%; and in France, 80.2 to 85.2% [18]). A higher prevalence (64%) was reported for a sample of housed flocks, also not subjected to partial depopulation, reared in England from 2000 to 2002 (10). Some European countries have reported decreases in *Campylobacter* prevalence since the late 1990s (4, 7, 24, 27, 32, 51). There may have been a reduction in flock prevalence of *Campylobacter* in the United Kingdom since the mid- to late 1990s compared to the period in which our data were collected, possibly associated with increased biosecurity measures, e.g., brought in to reduce flock infection with *Salmonella* spp. In United Kingdom retail chickens, *Campylobacter* spp. were detected in 83% of carcasses in studies from the late 1990s (33, 35), in 70% in 2005 (39), and in 64% in 2004 to 2006 (11). Numbers of human *Campylobacter* cases in England and Wales were higher in the late 1990s than in 2004 to 2006 (<http://www.hpa.org.uk/Topics/InfectiousDiseases/InfectionsAZ/Campylobacter/EpidemiologicalData/campyDataEw/>; accessed 7 January 2011). However, more recently, a prevalence of 75% was

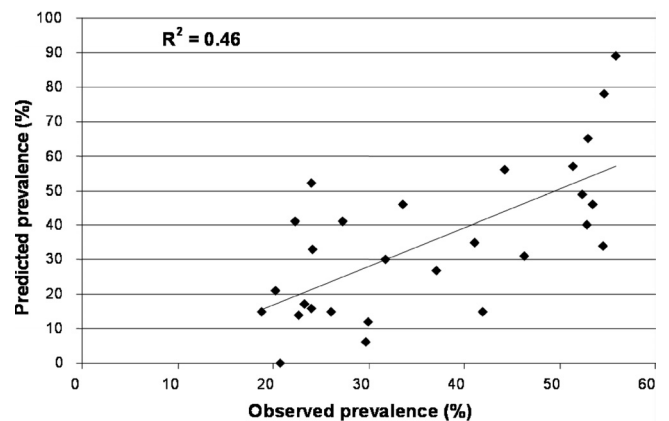


FIG. 4. Ability of climate factors (temperature at slaughter, sunshine hours in the month of placement, and rainfall in the month of placement) to predict the monthly *Campylobacter* prevalence in 797 broiler batches in Great Britain during 2004 to 2006.

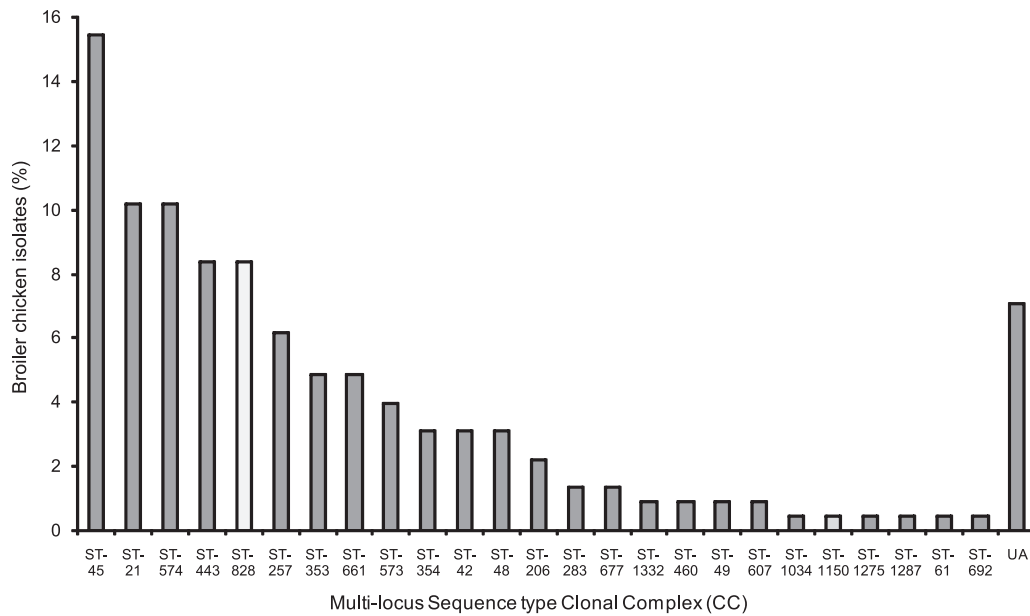


FIG. 5. Relative abundances of CC of *Campylobacter* spp. detected at slaughter in broiler flocks ($n = 226$) reared in Great Britain from 2003 to 2006. All isolates in CC with lighter shaded bars were *C. coli*. None of the flocks were previously partially depopulated. UA, sequence types not assigned to a CC.

found in a sample of 401 United Kingdom batches that included previously partially depopulated batches (20). Numbers of human *Campylobacter* cases in England and Wales were also higher in 2008 than in 2006.

The proportion of highly colonized flocks (~30%) was lower than that found in a study from 2001 where 75% of positive batches were *Campylobacter* positive at >70% (10). This could reflect late infection, e.g., associated with better biosecurity during rearing (9, 26), or slower spread of infection, e.g., related to treatment of drinking water (15). Most other studies have not tested several individual cecal samples within a batch for the presence of *Campylobacter* (5, 7, 18, 44, 53), but such data are useful for scientists designing risk assessment models to determine the most appropriate and effective intervention strategies to reduce *Campylobacter* prevalence in poultry. The within-flock prevalence affects the number of *Campylobacter* organisms on carcasses (2), and this significantly relates to the risk of contracting campylobacteriosis (45).

One of the best predictors of *Campylobacter* prevalence was whether flocks were reared during late summer and early autumn months or at other times of the year. This relationship between season and *Campylobacter* prevalence in broiler flocks was also observed in a geographical subset (46) of the data

examined in this work but was not detected in older studies from the United Kingdom, possibly because the data sets were smaller, the general incidence was higher, and/or the data sets included thinned batches (21, 28). However, a slightly higher prevalence during summer was detected in 2008 in 401 batches from the United Kingdom, and there was also a significantly higher prevalence in the summer months for the European Union overall (20). Previous studies have also found higher prevalence rates in the summer months in France (29, 44), the Netherlands (7, 31), Denmark (55), Iceland (5), Sweden (24), Norway (27), and Finland (32). Although the month cannot be the cause of *Campylobacter* colonization, it may be a good marker of the overall climate during the life cycle of the flock, and hence could be a proxy climate marker. There was a significant relationship between climatic factors (temperature at placement and average amounts of sunshine and rainfall) and *Campylobacter* prevalence which was able to explain 46% of the *Campylobacter* prevalence. This highlights that while climate is important, other factors related to broiler rearing, such as husbandry practices, biosecurity, or bird health, may also be important. The temperature 3 weeks before slaughter has been used to predict *Campylobacter* colonization of housed broiler flocks in Denmark, and while it was accurately predictive in some parts of the year, it was not so in the spring (43).

The mechanism by which seasonality/temperature affects *Campylobacter* colonization of broilers is unclear. It may be linked to changes in flock management and microbial survival and/or to greater ingress of wildlife vectors in warm weather. Housed broiler flocks are reared in environments where there is a degree of control, and as the temperature in a house rises, the ventilation rates and fan speeds increase in an attempt to compensate; thus, the airflow through the house increases. Climatic factors may also affect the sources of *Campylobacter* in the farm environment, such as insects and rodents. Temper-

TABLE 2. Multilocus STs, CC, and *flaA* SVR types for strain groups where 4 or more isolates had identical ST and *flaA* SVR types

CC	ST	<i>flaA</i> SVR	No. of isolates
574	574	105	14
45	45	8	7
443	51	316	6
257	257	16	6
48	48	32	4

ature may increase or decrease the survival of a vector, change the rate of its population growth, or change the way that the vector feeds or behaves (30). Fly populations in the United Kingdom are generally greater during the summer months, when conditions for fly development are optimal. Experimentally, house flies can transmit *C. jejuni* to broiler chickens (47), and *Campylobacter*-positive flies have been captured in a broiler house during the summer months (23). The presence of rodents on the farm can increase the risk of *Campylobacter* colonization of broiler flocks (6, 34), and rodent populations can increase during the summer months. Husbandry practices with other farm animals are also seasonal, and their presence may be associated with *Campylobacter* colonization of broiler flocks. Cattle especially appear to carry high levels of *C. jejuni*, and it is feasible that they may provide a reservoir for *Campylobacter* during down time between flocks (16). Rainfall may also contribute to presenting sources of *Campylobacter* in the farm environment by presenting persistent surface water reservoirs.

C. jejuni was much more common than *C. coli* in our flocks, and previous United Kingdom flock data also found this species more frequently than *C. coli* in housed broilers (9). We did find a considerable amount of diversity in *Campylobacter* genotypes in our data set. Nonetheless, the five most common clonal complexes in our study were the same as those commonly associated with chickens in a recent study from Scotland (48), although clonal complex ST-574 was more common and ST-257 was less common in our data set. The proportions of strains not assigned to a clonal complex were similar in both studies. In a recent study of isolates obtained from retail chicken in England, ST-45, ST-574, ST-51, and ST-257 were also among the five most common STs found (49). The most common clonal complexes found in the chicken flocks in this study have also been reported as the most common ones associated with human cases in the United Kingdom (14, 48, 56), although clonal complexes ST-661 and ST-573 were less common among isolates from human cases, and we detected very few clonal complex ST-61 and ST-206 isolates and no ST-658 isolates. This is consistent with ST-661 and ST-573 being associated mainly with chickens, while ST-61 and ST-206 have been associated with ruminants. While clusters were detected, they represented only a small proportion of the entire data set. Clonal complex ST-45 has previously been reported as seasonal in human cases and in recreational surface waters from N/NW England, possibly associated with transmission via direct exposure to water through outdoor activities (50). It was hypothesized that the seasonal presence of ST-45 in surface waters could relate to seasonal excretion from livestock and/or seasonal changes in husbandry factors. Our data found a significant seasonal pattern of the incidence of clonal complex ST-45 among flocks, with a peak incidence in June, and this could also suggest that seasonal contamination of chicken meat may affect seasonality of ST-45 in human cases.

Finally, we noted that a cluster of human *flaA* SVR105/ST574 cases were reported by Dingle et al. (14) at a similar time to that for which we found a cluster of this type, from when sampling began to September 2004, and that the majority (21/23 isolates) of the clonal complex ST-574 isolates also came from poultry reared in a similar area of Great Britain (the Midlands and central/southeast England).

In this work, we have demonstrated that there is an important effect of season on the prevalence of *Campylobacter* in broiler flocks that have not been partially depopulated. Although there is debate about the practicalities and cost implications of maintaining rigorous biosecurity, there is a general consensus within the scientific community that the number of positive flocks can be and has been reduced by these methods (1). It may be possible to apply enhanced biosecurity, along the lines of that in routine use in Scandinavia, at times of the year when the risk is greatest, such as the summer and autumn months.

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