Prevalence of *Campylobacter* spp. in Raw Retail Poultry on Sale in Northern Ireland

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ABSTRACT

A year-long survey of fresh, retail poultry products on sale in Northern Ireland was undertaken to define the prevalence of *Campylobacter* spp. by using protocols based on ISO (standard) 10272-1:2006. Incubation at 37 and 42°C was undertaken to increase the diversity of isolates obtained. Overall, 652 isolates were identified as *Campylobacter* spp. by using PCR and amplified fragment length polymorphic typing. Phenotyping wrongly identified 21% of isolates. Prevalences of *Campylobacter* found were chicken, 91% (n = 336); turkey, 56% (n = 77); and duck, 100% (n = 17). Prevalence rates for chicken produced in Northern Ireland, Scotland, England, and Wales were similar, with a mean value of 91%. The prevalences in product from the latter two countries were much higher than were found in two United Kingdom–wide surveys of chicken. The incubation temperature did not affect the relative proportions of the species isolated (P > 0.05). *Campylobacter jejuni* composed 64.6% of isolates, *Campylobacter coli*, 27.4%, and *Campylobacter lari*, 1%. Most cases of human campylobacteriosis are caused by *C. jejuni* and *C. coli*. The overall *Campylobacter* prevalence results are consistent with Northern Ireland surveys undertaken since 2000, and indicate that United Kingdom strategies to control *Campylobacter* in chicken have not had a significant effect on the prevalence of this pathogen in retail products on sale in Northern Ireland.

*Campylobacter* spp., especially *C. jejuni* and *C. coli*, are among the principal causes of gastroenteritis worldwide (5, 52). Raw chicken is frequently considered an important source of *Campylobacter* spp. (42), although *campylobacters* have also been isolated from other raw meats such as beef, pork, and lamb, as well as cooked meats and seafood (12, 25, 58, 61). Specific campylobacteriosis outbreaks have been identified as being caused by chicken (14), as well as at least one continuous source outbreak (42).

The economic costs of campylobacteriosis caused by retail poultry are large, with an estimate of €10.9 million (roughly US$14.4 million) for Belgium alone (15). In view of the problems caused by *Campylobacter* spp. in poultry products, there is still a need for research on these organisms in the food production environment (23). In 2001, the United Kingdom Food Standards Agency conducted a nationwide baseline survey of retail chicken to define the prevalence of *Campylobacter*, and found it to be 55% in fresh product (1). However, the laboratory responsible for Scottish samples reported 89% prevalence, as did the laboratory responsible for Northern Ireland samples. In contrast, the laboratory responsible for English and Welsh samples found prevalences of 52 and 47%, respectively. Subsequently, 83% prevalence has been reported in England (25) and 87% in Wales (31), suggesting the baseline survey data were not accurate. Since retail poultry on sale in Northern Ireland is imported from several countries (including Scotland, England, and Wales), local consumers could be exposed to a wide range of campylobacters, and there is a need to accurately define the prevalence.

Procedures to isolate *Campylobacter* spp. from food-stuffs have been defined by the International Organization for Standardization in ISO 10272-1:2006 (4), which is based on the use of Bolton enrichment broth incubated at 41.5°C to isolate the thermophilic campylobacters, which include *C. jejuni* and *C. coli*. Over recent years, there has been growing evidence that other *Campylobacter* spp. may also be human pathogens; however, they usually require an incubation temperature of 37°C for successful growth and isolation (13, 54), and hence would not be detected after incubation at 41.5°C. Further, the use of two incubation temperatures, 37 and 42°C, was recommended to maximize the genetic diversity of *Campylobacter* isolates from meats (50).

An investigation of ISO 10272-1:2006 for the isolation of campylobacters from raw chicken found that the incubation of enrichment cultures (using Bolton broth) required revision to maximize recoveries (33). Using the improved protocol, a survey was planned to accurately determine the prevalence of *Campylobacter* spp. in retail packs of fresh poultry on sale in Northern Ireland in order to gain information on the potential risk to the local population and to discover if it related to the country of origin of the meat. Duplicate samples were incubated at 42 and 37°C to determine if the latter temperature allowed the recovery of a wider range of campylobacters.

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MATERIALS AND METHODS

All media used were obtained from Oxoid, Ltd. (Basingstoke, UK), and all chemicals from Sigma-Aldrich (Poole, UK), unless otherwise stated.

Sample collection. A year-long survey of retail packs of chilled, raw poultry obtained from supermarkets and butcher shops in the greater Belfast area was undertaken, with packs purchased at a rate of approximately 10 per week. When packs were chosen in supermarkets, sample diversity was ensured by selecting a range of European Union (EU) processor codes and pack “sell-by” dates. A random selection of retail products was obtained, from whole birds to ground meat. Normally, only one sample was purchased in a butcher shop per visit, and only the “use-by” date was noted. All analyses were commenced within 2 h of purchase.

Campylobacter spp. isolation procedure. Enrichment procedures were undertaken at two temperatures, 37 and 42°C, to maximize recoveries. Samples were enriched in Bolton broth (CM0983 plus laked horse blood, SR0048, and supplements SR0183) by using a method based on ISO 10272:1:2006 and were shown to give equivalent results (32). Briefly, two subsamples of 10 g were aseptically excised from each pack, and each was blended in a stomacher blender (model 400, Seward, West Sussex, London, UK) at full power (1 min) with 90 ml of Bolton broth prior to microaerobic incubation (85% N₂, 10% CO₂ and 5% O₂) in a Scientific Mk III anaerobic cabinet (Don Whitley Scientific, Shipley, UK). One sub-sample was incubated for 24 h at 37°C, and the other incubated for 4 h at 37°C, then 20 h at 42°C. A loopful of each enrichment was streaked to single colonies on modified charcoal cefoperazone deoxycholate agar, and incubated at the temperature of the enrichment broth in a microaerobic atmosphere. Plates were examined after 24 and 48 h.

One typical Campylobacter colony per positive enrichment culture was streaked to purity and phenotypically identified by standard biotyping tests: motility, Gram stain, presence of catalase and oxidase, hippurate hydrolysis, and resistance to nalidixic acid and cephalothin (9, 54). From a plate of each purified isolate, a small piece (equivalent to a 10 µl loopful) was removed and placed in 1 ml of SET buffer composed of 150 mM NaCl, 15 mM EDTA, and 10 mM Tris-HCl, with a pH of 8.0 for DNA extraction. The remainder of each plate was harvested and stored in 1 ml of nutrient broth plus 10% (vol/vol) glycerol at −80°C.

Genotypic identification of genus and species. DNA was extracted from all isolates by using a phenol-chloroform method (21). Integrity and quantification of the DNA was visually estimated against lambda DNA standards in 2% agarose gels. The DNA solution was subsequently diluted to give a working concentration of 10 to 50 ng/µl and stored at −20°C.

Identification of the strains was conducted by using an automated amplified fragment length polymorphism (AFLP) method (24, 38) performed on an ABI Prism 3100 genetic analyzer (Applied Biosystems, Foster City, CA). Profiles were imported into BioNumerics v4.6.1 software (Applied Maths, Sint-Martens-Latem, Belgium) for analysis. The normalized AFLP profile similarities were calculated by using the Pearson product-moment correlation coefficient. Clustering and dendrogram construction were performed by the unweighted pair group with mathematical average method. DNA from known cultures of C. jejuni and C. coli was used for standards and included in each run. The profiles of the standards were analyzed separately and gave a similarity value of ≥90% for identical isolates with a position tolerance setting of 0.07% and a profile size range of 50 to 500 bp.

A subset of isolates was also identified by using the multiplex PCR method of (35), which produces amplicons specific to five pathogenic Campylobacter spp.: C. jejuni, C. coli, C. lari, C. upsaliensis, and C. fetus subsp. fetus. The assay also produces a characteristic amplicon from the 23S rRNA gene in the presence of any of the following: Campylobacter spp., Arcobacter spp., or Helicobacter pylori. Further identification, if required, was based on 16S rRNA sequencing (37), as modified by Harrington and On (20).

Where Arcobacter spp. were suspected, PCR was applied (7) (which yields a 331-bp amplicon indicative of Arcobacter). Confirmed Arcobacter isolates were speciated by the multiplex PCR method of Houf et al. (22).

The data on presence or absence of campylobacters were analyzed by regression, assuming a binomial distribution by using the GenStat for Windows, 11th edition (41). The model fitted included the effects of processor location and, for poultry, type of meat. When significant (P < 0.05) effects were found, the Student’s t test was used to test for pairwise differences between the effect levels. GenStat for Windows, 11th edition, was also used for statistical analyses.

RESULTS

Four hundred thirty retail packs of raw poultry were analyzed, with 88.6% being obtained from supermarkets, as they have the dominant market share. Purchasing in major supermarket chains also had the advantage that their use of centralized distribution meant that representative samples could all be obtained locally (29). Samples had a mean remaining shelf life of 2.6 days, with a minimum of 0 and a maximum of 7 days. A total of 687 presumptive Campylobacter spp. were isolated and phenotyped. The isolates were genotyped to species level by using AFLP (Table 1). The percentage of positive packs was not related to the remaining shelf life (P > 0.05). AFLP profiles showed a cluster of profiles distinct from Campylobacter spp. Further investigations indicated that Arcobacter butzleri had been isolated but only from enrichments incubated at 37°C. The AFLP speciation was confirmed by using the genus PCR (7) and species multiplex PCR (22).

Study of the AFLP profiles also suggested that some mixed cultures were present. The relevant cultures were again streaked to purity, with at least five colonies studied per culture, and this work yielded an additional six isolates.

<table>
<thead>
<tr>
<th>Table 1. Species of Campylobacteraceae isolated from retail packs of raw poultry, as determined by phenotyping and AFLP genotyping</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
</tr>
<tr>
<td>------------------</td>
</tr>
<tr>
<td>Campylobacter jejuni</td>
</tr>
<tr>
<td>C. coli</td>
</tr>
<tr>
<td>C. lari</td>
</tr>
<tr>
<td>C. upsaliensis</td>
</tr>
<tr>
<td>C. hyointestinalis</td>
</tr>
<tr>
<td>Arcobacter butzleri</td>
</tr>
<tr>
<td>Not Campylobacteraceae</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>
TABLE 2. Species of Campylobacteraceae isolated from retail packs of raw poultry at two incubation temperaturesa

<table>
<thead>
<tr>
<th>Species as defined by genotyping</th>
<th>37°C (%) of isolates</th>
<th>42°C (%) of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Campylobacter jejuni</td>
<td>206 (59)</td>
<td>242 (70)</td>
</tr>
<tr>
<td>C. coli</td>
<td>91 (26)</td>
<td>99 (28)</td>
</tr>
<tr>
<td>C. lari</td>
<td>1 (1)</td>
<td>6 (2)</td>
</tr>
<tr>
<td>Arcobacter butzleri</td>
<td>41 (12)</td>
<td>0</td>
</tr>
<tr>
<td>Unknown</td>
<td>7 (2)</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>346</td>
<td>347</td>
</tr>
</tbody>
</table>

a The differences in the distributions of Campylobacter spp. seen at the two temperatures were not significantly (P > 0.05) different.

Two isolates were found to be untypeable by AFLP, but sequencing of the 16S rRNA gene identified one as C. jejuni.

Phenotypic identification of 146 (21%) Campylobacter isolates conflicted with that given by the AFLP analysis; hence, all of these isolates, plus 148 others chosen at random, were analyzed by using multiplex PCR (55). The PCR results agreed with the AFLP speciation in all cases.

Comparison of the campylobacter recoveries obtained at the two incubation temperatures, 37 and 42°C (Table 2), showed the incubation temperature had no significant (P > 0.05) effect on the prevalence of Campylobacter spp. found, or in the relative proportions of individual species. C. lari was isolated from five samples of duck and two samples of turkey.

In total, 366 (85.1%) packs yielded Campylobacter spp. with 274 (63.7%) being positive at both incubation temperatures, while 70 (16.3%) were only positive at 42° C and 22 (5.1%) only positive at 37°C. Sixty-four (14.9%) packs did not yield any Campylobacter spp.

Three types of poultry were sampled: chicken (n = 336), turkey (n = 77), and duck (n = 17), and campylobacters were isolated from 91, 56, and 100% of packs, respectively. The prevalence of campylobacters in packs of turkey was significantly (P < 0.001) lower than the prevalence in chicken.

Samples from supermarkets carried 26 EU processor codes from six geographical regions, but ground meat did not require such a code. Hence, samples were obtained from eight sources, and the prevalence of Campylobacter spp. in chicken from these is shown on Table 3. After incubation at 42°C, 87% of chicken samples were positive, while at 37°C, 79% were positive. This difference is statistically significant (P < 0.001), but the lower prevalence at 37°C is probably a consequence of the growth of arcobacters, leading to reduced recoveries of campylobacters.

Overall, 41 packs yielded arcobacters, and from 33 of these, Campylobacter spp. were obtained at 42°C.

AFLP analysis revealed that for the 274 packs yielding campylobacters from both 37 and 42°C enrichments, genotypically identical isolates were only obtained from 30% (n = 83). In addition, 43% (n = 119) of these packs yielded the same species from both enrichments, but the isolates had different genotypes.

TABLE 3. Prevalence of Campylobacter spp. in retail packs of raw chicken, according to the sample sourcea

<table>
<thead>
<tr>
<th>Sample source</th>
<th>No. of EU processor codes</th>
<th>No. of samples</th>
<th>Positive samples (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ground meat</td>
<td>NAb</td>
<td>7</td>
<td>86</td>
</tr>
<tr>
<td>Butcher</td>
<td>NA</td>
<td>42</td>
<td>93</td>
</tr>
<tr>
<td>Republic of</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ireland</td>
<td>2</td>
<td>2</td>
<td>100</td>
</tr>
<tr>
<td>Scotland</td>
<td>1</td>
<td>17</td>
<td>100</td>
</tr>
<tr>
<td>France</td>
<td>1</td>
<td>6</td>
<td>100</td>
</tr>
<tr>
<td>Northern</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ireland</td>
<td>4</td>
<td>166</td>
<td>91</td>
</tr>
<tr>
<td>Wales</td>
<td>3</td>
<td>9</td>
<td>89</td>
</tr>
<tr>
<td>England</td>
<td>15</td>
<td>87</td>
<td>89</td>
</tr>
<tr>
<td>Total</td>
<td>26</td>
<td>336</td>
<td>91</td>
</tr>
</tbody>
</table>

a In supermarkets, ground chicken did not require a EU processor code; hence, these results are reported separately. No data on the source of samples were obtained from butcher shops.

b NA, not applicable.

DISCUSSION

The specific genotypes of Campylobacter spp. obtained from raw poultry are significantly affected by the incubation temperature used (50); hence, in this study, duplicate samples were incubated at both 37 and 42°C to maximize diversity. Isolates were initially identified by a basic phenotyping scheme and then genotyped to confirm the speciation. The limited biochemical activity of Campylobacter spp. means that their identification by using phenotyping alone is problematic; thus, genotyping should also be applied (34, 51).

Of the 687 isolates obtained in this study, phenotyping gave erroneous results for about a fifth of the isolates obtained, confirming the need for genotyping to ensure accurate identification. One specific problem was that 41 isolates obtained at 37°C were A. butzleri, but these were phenotyped as C. coli. Arcobacters grow aerobically (34), unlike campylobacters. However, A. butzleri is often microaerobic on initial isolation (11); thus, this test can provide misleading results. Since arcobacters, but not campylobacters, exhibit microaerobic growth at 25°C, this test would be more useful than would evaluating aerobic growth when only limited phenotyping studies are performed.

Incubation at 37°C did not yield a wider range of Campylobacter spp. than did incubation at 42°C (Table 1). Since Arcobacter spp. do not grow at 42°C (54), the use of this temperature would simplify the isolation of Campylobacter spp. from poultry by eliminating a genus capable of causing false-positive results.

In this study, 91% of retail packs of chicken were found to carry campylobacters, and there was no correlation between the declared location of the poultry processing plant and the prevalence of campylobacters, with samples from all parts of the United Kingdom showing 89 to 100% contamination. In the United States, 71% prevalence has been reported (61), and in Belgium, 72% (16). In the
Australian states of New South Wales and South Australia, prevalences of 87.8 and 93.2%, respectively, were reported (44), very similar to our findings (Table 3). In Turkey, 83.4% of retail chicken meat carried Campylobacter spp. (49), while English studies found 79% (10) and 83% (25), and a Scottish study found 90% were infected (17). Thus, the high prevalence reported in this study is not unusual when compared with both United Kingdom and international findings.

Our results are also in agreement with the findings of two surveys of whole chickens conducted in Northern Ireland. Meldrum and Wilson (31) found 86.2% of Northern Ireland retail chickens carried campylobacters, and Moore et al. (32) found 94% carried campylobacters. Hence, retail chicken in Northern Ireland has shown a consistently high prevalence of Campylobacter spp. over a period of several years, and based on these observations, the United Kingdom Food Standards Agency’s pledge to reduce the incidence of Campylobacter by 50% in United Kingdom–produced chickens (3) by 2010 is unlikely to be met.

However, because a wide range of media and methods have been used to isolate Campylobacter spp. from foodstuffs, and methodologies have improved significantly over time, comparison of the results of different studies must be undertaken with caution. For example, in Belgium, a study of broiler carcasses was undertaken from 1997 to 1998 and found 25.6% of carcasses carried campylobacters (53), but a second investigation, conducted in 1997 and 1998, found 71.9% (n = 270) to be positive (16). Further, in the United States, the prevalence of Campylobacter spp. on broiler carcasses was reported as being highly seasonal, being up to 97% in summer but falling to 7% in December (59). A study of retail chicken meat preparations in Belgium (18) also found evidence of prevalence seasonality, but from approximately 35 to 70%, peaking in July. Thus, surveys conducted over less than a year could give skewed results.

A major United Kingdom survey of retail raw chicken (n = 1,778) found that 61% of samples carried campylobacters (27), very similar to the 60% found in a comparison of methodologies by using a similar protocol (33). However, the latter study also found that if enrichments were incubated in a microaerobic atmosphere, rather than in closed bottles, then 93% of samples were positive. Incubating chicken samples in Bolton broth in closed bottles was also reported as causing recoveries of campylobacters inferior to microaerobic incubation (18). Thus, once again, the comparison of survey results must be undertaken with care, since minor methodological differences can markedly affect prevalences found.

Considering the relative proportions of the species found, these species could be markedly affected by the enrichment medium used (28). However, in studies of raw chicken, C. jejuni is normally the dominant species, with C. coli making up a minor proportion of the flora and C. lari only occasionally isolated (8, 27). A Japanese study of chicken (48) found 82% of isolates were C. jejuni and 18% C. coli, similar to the respective figures of 85 and 15% found in an Irish study (58). However, a U.S. study utilizing Bolton broth, as did this study, found 52% of chicken samples to be positive, with almost equal numbers of C. jejuni and C. coli obtained (39).

Considering only the isolates from the 42°C incubations in this study (Table 2), 70% were C. jejuni and 28% C. coli, very similar to the respective figures of 69 and 30% found for an earlier study of retail chicken in Northern Ireland (32). A Turkish study also reported 70% C. jejuni, but found 21% C. coli and 9% C. lari (49); the latter species was only a minor (2%) part of the Campylobacter flora in this study.

C. jejuni is the dominant cause of campylobacteriosis in humans, with C. coli accounting for less than 10% of cases and C. lari for less than 1% (47, 56). Hence, the latter two species may be less pathogenic than is C. jejuni, and chicken contaminated with C. coli or C. lari might pose less of a health risk than if C. jejuni were present. However, 20% of Campylobacter-positive packs yielded two species, despite only one colony from each enrichment being identified. Thus, a significant number of packs of chicken in Northern Ireland will carry two species of Campylobacter, and consumers will consequently be exposed to these organisms.

In this study, although at most two isolates per pack were analyzed, genotyping of the campylobacters revealed that where two isolates were obtained from the same pack, they were different in 70% of cases. Thus, it was common for packs to be contaminated by more than one species and/or genotype. Studies on broiler carcasses have revealed that it is common for multiple Campylobacter genotypes to be present (26); hence, it would be expected that retail packs would be similarly contaminated, especially given the opportunities for cross-contamination during processing (35, 36). Illness in humans caused by chicken has been seen to result in coinfection by two strains of C. jejuni (14), and such coinfections pose problems for epidemiological investigations (46).

Only 17 duck samples were analyzed in this study, and all carried campylobacters. A prevalence of 73% in wild ducks was reported (40), while highly variable results (from 3 to 60% of birds) were found in three flocks of farmed ducks (30). Therefore, these birds can carry campylobacters, and given the mechanized nature of poultry processing, many opportunities for cross-contamination can occur, leading to the high prevalence found. Few studies of duck meat have been published, and given the small number of samples studied, there is no detailed consideration of this result here. However, unlike chicken, duck may be consumed rare (44); therefore, campylobacters may constitute a particular hazard with this meat.

Turkey samples showed significantly lower campylobacter prevalence than did the other two poultry meats in this study, 56%. A carriage rate of 87% (60) in turkeys was found, although rates have been reported as varying from 65 to 90% among flocks (56). High prevalence in retail meats could therefore be expected, and 79% was reported for skinless breast fillets in Germany (19). However, another German study of retail turkey meats found only 34% (6). A United Kingdom survey of retail meats also reported that 34% (n = 214) of turkey samples carried campylobacters.
(27), but that study was based on incubating enrichment cultures in sealed bottles, which significantly reduces recoveries of campylobacters (18, 33).

Given the desire of the United Kingdom government to reduce the incidence of human campylobacteriosis (2), further study of Campylobacter spp. in the production of turkey meat to determine the cause of the significantly lower Campylobacter prevalence may yield useful information.

Overall, a survey of poultry meats on retail sale in Northern Ireland revealed that incubation of the enrichment medium, Bolton broth, at 42 °C rather than 37 °C, prevented the growth of Arcobacter spp. but did not affect the range of Campylobacter spp. found. The prevalence of campylobacters in chicken and duck was more than 90%, but it was lower in turkey. The prevalence in chicken, 91%, was in accord with Northern Ireland surveys reported since 2000 and with some international studies, but was significantly higher than found in two United Kingdom national surveys. Based on the consistently high prevalence of campylobacters found in Northern Ireland retail chicken, and the fact that supplies are brought from throughout Britain, the United Kingdom government goal of reducing the prevalence of Campylobacter in United Kingdom chicken by 50% between 2005 and 2010 (3) will be difficult to achieve.

The Campylobacter spp. found were predominantly C. jejuni (69%) and C. coli (29%), both of which are major causes of foodborne illness in humans. A significant proportion of packs of chicken were contaminated by more than one species and/or more than one AFLP genotype of Campylobacter. Thus, most retail poultry on sale in Northern Ireland may have the potential to cause human illness if not handled appropriately.

REFERENCES


