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Occurrence of *Campylobacter* in retail foods in Ireland

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Abstract

A surveillance study was carried out to determine the prevalence of *Campylobacter* in a range of retail foods purchased in three Irish cities over a 20-month period between March 2001 and October 2002. In total 2391 food samples were analysed during this period. *Campylobacter* was isolated from 444 raw chicken (49.9%), 33 turkey (37.5%) and 11 duck samples (45.8%). Lower isolation rates of 7/221 (3.2%), 10/197 (5.1%) and 31/262 (11.8%) were observed for raw beef, pork and lamb, respectively. One sample of pork paté from 120 samples analysed (0.8%) was *Campylobacter*-positive. A total of three shellfish samples (oysters) from 129 raw specimens examined (2.3%) were found to contain *Campylobacter*. Low prevalences of the organism (0.9%) were also isolated from fresh mushrooms. Of 62 raw bulk tank milk samples analysed, *Campylobacter* was recovered in a single sample (1.6%). *Campylobacter* was not detected in any of the comminuted pork puddings, prepared vegetables and salads, retail sandwiches or cheeses made from unpasteurised milk. In total, 543 *Campylobacter* were isolated from all of the food samples analysed, of which 453 (83.4%) were confirmed as *Campylobacter jejuni* and the remaining 90 (16.6%) as *Campylobacter coli*.

Keywords: *Campylobacter*; Prevalence; Food; Contamination; Isolation

1. Introduction

Disease in humans as a result of consuming pathogen contaminated foodstuffs remains a significant burden on society, causing suffering and

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economic losses in terms of decreased productivity and medical costs (Bryan and Doyle, 1995). Food is now recognized as the most frequently implicated vehicle in the transmission of zoonotic organisms to humans in developed countries (Jorgensen et al., 2002). Of these pathogens, *Campylobacter* is the principal bacterial cause of gastroenteritis in many developed countries, including Ireland (Nachamkin et al., 1998; PHLS, 2001; Fitzgerald et al., 2001). The National Disease Surveillance Centre (NDSC) reported 2085 human cases of campylobacteriosis in Ireland in 1999; this was equivalent to a crude incidence rate of 57.5 cases per 100,000 population (Whyte and Igoe, 2000). The reported annual incidence rate for human *Campylobacter* infections in other European Union countries has varied between 9.5 and 108 cases per 100,000 people in 1997 (Anon., 1999).

A number of transmission vehicles and risk factors have been implicated in previously reported case control studies that examined predisposing data from human *Campylobacter* cases and outbreaks. The most significant risk factors identified have included the consumption and/or handling of raw or undercooked poultry or other meats, raw milk and surface waters. Cross-contamination of ready to eat foods during food preparation as well as direct contact with animals have also been identified (Anon., 1994; Tompkin, 1994; Adak et al., 1995). It is now well established that animals can be asymptomatic intestinal carriers of *Campylobacter* and foods of animal origin can become contaminated by this pathogen during slaughter and carcass dressing (Berndtson et al., 1996; Madden et al., 2000; Whyte et al., 2003). Furthermore, it is now accepted that campylobacteriosis is predominantly acquired through the consumption of contaminated foods (Anon., 1995).

Wide variations in the prevalence of *Campylobacter* have been reported in both live animals and foods of animal origin. For example, previously reported infection rates in live broilers have ranged from 0% to 100% (Bryan and Doyle, 1995; Moore et al., 2003) with high prevalences of up to 100% also in pigs (Nesbakken et al., 2003) and up to 60% in cattle (Orr et al., 1995; Nielsen et al., 1997). *Campylobacter* prevalences of up to 100% have also been reported on dressed poultry carcasses (Waldroup et al., 1992; Atanassova and Ring, 1999; Dominguez et al., 2002) with significantly lower prevalences of the organism generally reported on either beef or pork carcasses (Kwiatek et al., 1990; Zanetti et al., 1996; Madden et al., 2001). Other foods that *Campylobacter* has been recovered from include raw milk (Rohrbach et al., 1992; Leclerc et al., 2002), and shellfish (Wilson and Moore, 1996; Endtz et al., 1997).

The current research was carried out to address the lack of data pertaining to the prevalence and types of *Campylobacter* spp. found in retail foods widely available throughout Ireland. The aim of the study was to identify the principal food categories which represent the most significant reservoirs of *Campylobacter*.

2. Materials and methods

2.1. Sample collection

A survey of *Campylobacter* prevalences in a range of retail foods was undertaken in three population centres on the island of Ireland. The three cities selected were Dublin, Galway and Belfast with samples obtained from a number of retail outlets in each area. Samples were collected monthly from each centre from March 2001 to October 2002. These cities were selected based on their geographical location in order to establish whether prevalences varied between location for the various food types investigated. Samples were collected from both large retail outlets and smaller units including, dedicated butcher shops. In most instances, the meat samples purchased for the study were pre-packed supermarket products, with a smaller number of open or loose samples acquired from butchers. Samples were shipped to the laboratory on the day of purchase, in insulated bags containing cooled ice packs, and analyzed within 24 h of purchase. A range of foods were analyzed, including chicken, turkey, duck, pork, beef, lamb, shellfish (mussels and oysters), raw milk (from on-farm bulk tanks), prepared salads and vegetables, mushrooms, unpasteurized cheeses and ready-to-eat sandwiches. Approximately 30–35 samples in total were purchased and analyzed monthly from each city. A total of 2391 food samples were screened for the presence of *Campylobacter* during the course of the surveillance project.

2.2. Sample preparation

For solid foods such as meat or poultry, 10 g from each sample was aseptically removed using sterile scissors and forceps. Samples were placed in 90 ml volumes of Preston broth (Mast Diagnostics, Merseyside, UK and Oxoid, Basingstoke, UK) in sterile plastic bags and processed for 1 min in a stomacher (Lab Blender 400, Seward Medical, London, UK). The stomached samples and broths were then placed in sterile plastic disposable 100 ml universal containers and additional broth added, as required, in order to minimize head spaces within the bottles as recommended by Roberts et al. (1995).

Liquid food samples, including raw milk were selectively enriched by adding 50 ml volumes of sample to sterile sample bottles containing an equal volume of double strength Preston broth.

2.3. Microbiological analysis

Preston broths were made up according to the formulation developed by Bolton and Robertson (1982) and included growth and antimicrobial selective supplements as well as 5% v/v lysed horse blood. Following processing in a stomacher, all samples were selectively enriched in the Preston broths for 48 h at 42±1 °C. All enriched samples were subsequently subcultured on to selective solid media, modified charcoal cefoperazone deoxycholate agar (mCCDA, Mast Diagnostics and Oxoid). The mCCDA plates were incubated for 48 h at 42±1 °C under a microaerophilic atmosphere which was achieved using catalyst-free gas packs (Biomerieux, Mary l'Etoile, France). These isolation media and conditions have been frequently reported in other studies (Petersen et al., 2001; Nye et al., 2001; Wang et al., 1999). Suspect colonies on solid media were subcultured on to Columbia blood agar containing 5% v/v horse blood which were again incubated for 48 h at 42±1 °C in a microaerophilic atmosphere. Colonies were examined morphologically and Gram stained as presumptive identification of positives. Final confirmation and speciation was carried out using the CampID biochemical profiling system (Mast Diagnostics). The confirmation and identification of isolates was based on characteristic reactions for hippurate hydrolysis, indoxylacetate hydrolysis and urease activity.

2.4. Statistical analysis

The *Campylobacter* recovery rates from food samples obtained in all three cities were compared statistically using chi-square analysis with significance defined at the $p \leq 0.05$ level. All statistical analyses were carried out using Statview version 5.0.1 (SAS Institute, North Carolina, USA).

3. Results and discussion

The prevalence of *Campylobacter* isolated from all of the various food categories examined are summarized in Table 1. In the current prevalence study, 2391 food samples from a wide range of food categories were screened for the presence of *Campylobacter*. Of the foods sampled, 543 were found to be contaminated with *Campylobacter*. The most prevalent species recovered from samples was *Campylobacter jejuni*, with 83.4% of the isolates confirmed. The remaining 16.6% of isolates were identified as *C. coli*. With the exception of raw chicken, no significant differences in the prevalence of *Campylobacter* were observed between the three cities for any of the foods of animal origin examined (Table 1).

Poultry samples were most frequently contaminated with this enteropathogen with mean isolation rates from the three population centres of 49.9%, 37.5% and 45.8% from broiler, turkey and duck samples, respectively. All of the poultry samples purchased were domestically produced on the island of Ireland. It is noteworthy that isolation rates of *Campylobacter* in chicken samples from Galway were significantly lower compared to Dublin samples ($p \leq 0.05$). Previously reported prevalences in fresh processed poultry have varied widely between 0% and 90% (Jacobs-Reitsma, 2000; Cloak et al., 2001; Jorgensen et al., 2002). A survey conducted under the supervision of the U.K. Food Standards Agency found that an average of 50% of retail carcasses were contaminated with *Campylobacter* with mean prevalences ranging from 46%, 42%, 75% and 77% for England, Wales, Scotland and Northern Ireland, respectively (Anon., 2001). In another study carried for the Food Safety Authority of Ireland, it was found that 53% of raw poultry samples were *Campylobacter*-positive (Anon., 2002). These findings are in close agreement with the

Table 1
Prevalence of *Campylobacter* in a range of retail foods sampled in three Irish population centres

Food category	Sampling location			*Campylobacter* spp. isolated	Total
	A[a]	B[b]	C[c]		
Chicken	207/376[1] (55)	104/222 (46.8)	133/292[2] (45.5)	376 *C. jejuni* (84.7) 68 *C. coli* (15.3)	444/890 (49.9)
Duck	8/18 (44.4)	3/6 (50)	–	9 *C. jejuni* (81.8) 2 *C. coli* (18.2)	11/24 (45.8)
Turkey	12/24 (50)	17/54 (31.4)	4/10 (40)	28 *C. jejuni* (84.8) 5 *C. coli* (15.2)	33/88 (37.5)
Lamb	14/100 (14)	7/82 (8.5)	10/80 (12.5)	27 *C. jejuni* (87.1) 4 *C. coli* (12.9)	31/262 (11.8)
Pork	6/101 (5.9)	2/73 (2.7)	2/23 (8.6)	1 *C. jejuni* (10) 9 *C. coli* (90)	10/197 (5.1)
Beef	2/103 (1.9)	4/83 (4.8)	1/35 (2.8)	6 *C. jejuni* (85.7) 1 *C. coli* (14.3)	7/221 (3.2)
Seafood[d]	3/117 (2.5)	–	0/12 (0)	3 *C. jejuni* (100) –	3/129 (2.3)
Raw milk	0/10 (0)	1/52 (1.9)	–	– 1 *C. coli* (100)	1/62 (1.6)
Mushrooms	1/90 (1.1)	0/77 (0)	1/50 (2)	2 *C. jejuni* (100) –	2/217 (0.9)
Pork paté	1/53 (1.8)	0/42 (0)	0/25 (0)	1 *C. jejuni* (100) –	1/120 (0.8)
Pork pudding	0/19 (0.0)	–	0/4 (0.0)	–	0/23 (0.0)
Unpasteurized cheese	0/62 (0.0)	–	0/4 (0.0)	–	0/66 (0.0)
Vegetables/salad	0/46 (0.0)	–	0/16 (0.0)	–	0/62 (0.0)
Sandwiches	0/20 (0.0)	–	0/10 (0.0)	–	0/30 (0.0)
Total no. sampled	1139	691	561	453 *C. jejuni* (83.4) 90 *C. coli* (16.6)	543/2391

Results expressed as the number of *Campylobacter*-positive samples/total number of samples analyzed.
( ) = % samples positive.
[1,2]Different superscripts denote statistical significance between population centres ($p \leq 0.05$).
All samples obtained monthly from each location from March 2001 to October 2002.
[a] Location A=Dublin.
[b] Location B=Belfast.
[c] Location C=Galway.
[d] Seafood samples comprised oysters and mussels.

prevalences observed on raw retail poultry sampled for the current study. Speciation of the isolates confirmed that *C. jejuni* was the most prevalent species identified from poultry samples with 84.7%, 84.8% and 81.8% of isolates from chicken, turkey and duck recovered, respectively. All of the remaining isolates from these samples were identified as *C. coli*. Other studies have reported prevalences of *Campylobacter* species on raw poultry with the levels of *C. jejuni* recovered ranging from 80% to 98% which are in agreement with the current investigation (Kwiatek et al., 1990; O'Sullivan et al., 2000; Nielsen et al., 1997; Jorgensen et al., 2002). Variations in the prevalences of *Campylobacter* isolated from raw poultry reported in other studies may be a result of different sampling techniques employed, seasonality and laboratory methodologies employed.

Campylobacter was recovered at lower prevalences in retail meat samples from other food animal species, with isolations of 3.2% (beef), 5.1% (pork) and 11.8% (lamb) observed. *C. jejuni* was the most prevalent *Campylobacter* species recovered from beef and lamb samples with 85.7% and 87.1% of isolates confirmed as *C. jejuni*, respectively. The remaining isolates

recovered from these samples were confirmed as *C. coli*. Conversely, the dominant species observed in retail pork samples was *C. coli* (90%) with the remainder of isolates confirmed as *C. jejuni* (10%). Some processed foods of animal origin were examined, including pork patés and puddings. From these, *Campylobacter* was recovered from a single jar of paté of 120 sampled (0.8%). No viable *Campylobacter* was recovered from pork-based puddings (a comminuted pork product also containing grain and dry seasonings). Pigs and pork carcasses have been recognized as a reservoir for *Campylobacter*, particularly *C. coli*. Madden et al. (2000) recovered *Campylobacter* from 100% of *postmortem* anal swabs from pigs while Nielsen et al. (1997) reported a prevalence of 46% in pig faeces. Other studies have reported varying levels of *Campylobacter* isolation from pork ranging from 0% to 56% (Epling et al., 1993; Zanetti et al., 1996; Ono and Yamamoto, 1999; Pezzotti et al., 2003). The dominant *Campylobacter* species isolated from pork was *C. coli* which is in agreement with the current study. The prevalence of *Campylobacter* in beef samples was relatively low at 3.2%. Other studies have also demonstrated low prevalences in beef, with 0%, 0.9%, and 1.3% isolated by Ono and Yamamoto (1999), Kwiatek et al. (1990) and Pezzotti et al. (2003), respectively. In the present study, 11.8% of retail lamb samples were found to be *Campylobacter*-positive; these levels are higher than previously reported data (Edwards, 1999; Raji et al., 2000). Higher prevalences may have resulted from cross-contamination during boning and packing, particularly in small butcher shops where there may have been closer proximity to meat from other food animal species.

A total of three shellfish samples were found to be positive for *Campylobacter* which corresponded to 2.3% of the raw samples screened in this food category. The seafoods examined in our study were all raw specimens. All three isolates were subsequently confirmed as *C. jejuni* and all were found in raw oysters, with no *Campylobacter* organisms detected in any of the other seafoods tested. The consumption of contaminated shellfish have been implicated in foodborne illness (Abeyta et al., 1993). Higher prevalences of *Campylobacter* to those obtained in the current study have been reported previously in shellfish. Endtz et al. (1997) recovered the organism from 69.5% and 26.8% of batches of mussels and oysters sampled in The Netherlands. In a report from Northern Ireland, Wilson and Moore (1996) found *Campylobacter* in 42% of shellfish tested. It would appear that the levels of contamination in shellfish is directly associated with the microbiological quality of waters in which shellfish are cultured and harvested.

A low prevalence of *Campylobacter* was also observed in retail mushrooms with 2 samples found to be positive from 217 batches sampled (0.9%) which contrasted with the findings of McMahon and Wilson (2001), who did not recover the organism from fresh organic mushrooms. Both mushroom *Campylobacter* isolates recovered in the current study were confirmed as *C. jejuni*.

Transmission of *Campylobacter* infections to humans via the consumption of raw milk is acknowledged with numerous outbreaks and cases previously reported (Finch and Blake, 1985; Hargrett-Bean et al., 1988). *Campylobacter* was detected in one bulk tank raw milk sample out of a total of 62 examined (1.6%) in our study. The isolate was speciated as *C. coli*. Previous studies have also recovered *Campylobacter* from raw milk with prevalences up to 12.3% reported (Humphrey and Hart, 1988; Rohrbach et al., 1992). In Ireland, all retail liquid milk must be pasteurized as a minimum heat treatment. Therefore, most of the public would not be exposed to contaminated raw milk; however, the consumption of raw milk by farm families is still widespread and could pose a potential risk to public health.

Campylobacter was not isolated from ready-to-eat retail sandwiches, prepared salads and vegetables or cheeses made from unpasteurised milk. These foods would be unlikely to be contaminated with *Campylobacter* unless they acquired the organism during preparation as a result of cross-contamination. Other studies have also failed to detect *Campylobacter* in vegetables and unpasteurised cheeses (McMahon and Wilson, 2001; Allmann et al., 1995).

No apparent pattern in the seasonality of *Campylobacter* prevalences was observed in our study. Prevalences found in chicken sampled in all three cities varied greatly between seasons and ranged from 28.5% to 70.4%. In Europe, it is recognized from public health surveillance data that the numbers of human campylobacteriosis cases peak during the summer months (Altekruse et al., 1999; Sopwith et

al., 2003). Furthermore, other studies have shown an increase in the prevalence of infected live broiler flocks during these months (Altekruse et al., 1994). In the current study, seasonal peaks were not observed in retail chicken samples. It is suggested that the effect of seasonality observed in live birds may have been negated as a result of extensive cross-contamination during slaughter and processing, resulting in irregular and frequently high levels of positive samples year round.

Limited information has been available in Ireland pertaining to the prevalence of *Campylobacter* in retail foods. The purpose of our study was to investigate the prevalence of this enteropathogen in a wide range of foods available at retail level. In our study, it was found that foods of animal origin, and in particular raw poultry meat was most frequently found to be contaminated with campylobacters. This surveillance data will subsequently enable public health professionals to identify high risk foods and inform susceptible populations of the risks and any necessary precautions required. It will also enable more effective monitoring and surveillance systems to be developed which could target specific food industry sectors. Once identified, more stringent controls could be applied in high risk agri-food sectors at both pre-harvest and harvest levels through the application of longitudinally integrated safety assurance systems using HACCP principles.

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