Thermotolerant *Campylobacter* in poultry meat marketed in the Abruzzo and Molise regions of Italy: prevalence and contamination levels

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Summary

The prevalence and level of contamination of *Campylobacter* were determined for poultry meat taken from small and large retailers in Abruzzo and Molise. Of a total of 392 samples analysed, 160 (40.8%) revealed low-level thermotolerant *Campylobacter* contamination (0.3-9.3 most probable number [MPN]/g), 17 samples (10.6%) showed a contamination level above 9.3 MPN/g and one sample (0.6%) had 110 MPN/g. *Campylobacter jejuni* and *C. coli* were isolated from 81.9% and 32.5% of the samples, respectively. More than one species of *Campylobacter* were isolated from 23.1% of the positive samples.

Keywords

Campylobacter spp., Italy, Poultry, Food safety, Surveillance.

Introduction

In the European Union (EU) the incidence of thermotolerant *Campylobacter* infection in humans continues to increase (15, 16). The number of cases has now overtaken that for *Salmonella* species (16). *Campylobacter jejuni* is the most common cause of campylobacteriosis encountered in the European Union, followed by *C. coli* (16, 28, 30). In the United States in 2004, the incidence of infection in 100 000 people was 12.9 (8), while in the EU in the same year the rate was 41.3 cases per 100 000 people (16). There is no official data on the real incidence of infection in Italy, as data on cases of gastroenteritis caused by *Campylobacter* are not distinguished from those caused by other infections listed in class IV of the Ministerial Decree of 15 December 1990 'Infections, toxic infections and infestations of animal origin' (4). Surveys conducted in the Pesaro province from 1985 to 1992 on stool samples taken from patients with diarrhoea confirmed the presence of *Campylobacter* spp. in 2.3% of samples (6). In other surveys conducted from 1981 to 1990 (20) and in 1992 (7), *Campylobacter* was found in 10.8% and 7.9%, respectively, of children suffering from diarrhoea.

Campylobacter jejuni is also responsible for other extraintestinal forms (meningitis, peritonitis, pancreatitis, urinary infections, neonatal sepsis, miscarriage), and some chronic immunomediated diseases (endocarditis, nodal fever, reactive arthritis). Guillain-Barré syndrome (GBS), a neurological, post-infective form, is also associated with *C. jejuni* infection (3). Case-control serological studies have demonstrated a *C. jejuni* prevalence varying from 15 to 66% in subjects affected by GBS (1).

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Studies to identify risk factors have confirmed that contact with animals and the consumption of contaminated food (poultry meat, raw milk and contaminated water) are the principal sources of human campylobacteriosis. The most frequent source of human infection is the consumption of poultry meat. The risk increases when products are consumed away from home (2, 19, 32).

Surface contamination of poultry meat may occur during slaughter, but is more common in the scalding, defeathering and evisceration phases, which facilitate the transmission of microorganisms from one carcass to another (23, 31). The formation of contaminated aerosols during the defeathering phase is the source of contamination of not only the carcasses (21) but increases the risk of slaughterhouse workers contracting infection (15, 31).

Poultry meat has been incriminated as being responsible for 20-40% of all sporadic cases of infection in several countries (1, 28, 35). Recent microbiological surveys performed in the United States (1999-2000) and in the United Kingdom (1998-2000) on raw poultry meat taken from retail stores demonstrated *Campylobacter* spp. contamination of 70.7% and 83.0% respectively of all analysed samples (25, 37).

In Italy, the two studies conducted from 2000-2001 (Friuli Venezia Giulia, Trentino Alto Adige and Veneto) (33) and in 2001 (Forlì and Rimini Provinces) (9) found 35.71% to 81.3% *Campylobacter* spp. contamination, respectively. These results refer to analysis of meat samples taken at the end of the slaughtering and sectioning phases.

Levels of *Campylobacter* contamination reported in the literature show a wide range of values which, although generally low, are not comparable as they are affected by different sampling methods (18) and by the different poultry samples analysed (27). A 2001 survey in the United Kingdom found that 63.7% of poultry samples may have a contamination level of less than 2 log per examined portion (18). Other surveys found a contamination level of 1-10 colony-forming units (cfu)/cm^2, corresponding to about 2-20×10^4 cfu per carcass (27).

In Italy, data on the prevalence of *Campylobacter* spp. contamination in poultry products are insufficient and there are no reliable data on thermotolerant *Campylobacter* contamination levels in retail chicken meat.

This study was conducted to estimate the level of thermotolerant *Campylobacter* contamination in poultry meat marketed in Abruzzo and Molise to provide the data required to assess the risk of *Campylobacter* infection by consumers of poultry meat. This study will provide data to estimate the probability of human exposure to *Campylobacter*. A dose response model will be required in order for the exposure data to be translated into a risk estimate of human illness. The goal of this work was the estimation of *C. jejuni* level contamination in poultry products marketed in the Abruzzo and Molise regions which is one of the steps necessary to achieve the data necessary for risk assessment.

Materials and methods

The survey was conducted between December 2002 and June 2003. A total of 392 raw samples were taken from whole and sectioned chickens. Chicken pieces, deboned pieces, minced and chopped meat and preparations using additives (spices, flavourings, etc.) were excluded. The prevalence of infection was estimated based on the following: expected prevalence 50%, maximum error 5% and confidence level 95%. The choice of 50% expected prevalence was justified by both literature data (*C. jejuni* isolation between 38.8% and 88%) (10, 11, 25) and the lack of knowledge of actual prevalence in the European countries surveyed.

Due to the lack of up-to-date information on the sale and consumption of poultry meat in the provinces of Abruzzo and Molise, samples were divided by province in proportion to the resident population (from ISTAT 2001 data) (29) and the type of distribution channel. Samples were taken from retail stores selected from the computerised records of phone users in the two regions.

Retailers were divided into two distribution channels as established by Law No. 114 of 31 March 1998: large (super and hypermarkets, hard discounts) and small-scale distribution channels (butchers, etc.) (5).

A total of 392 samples of raw chicken meat were tested, of which 291 (74%) were taken in Abruzzo and 101 (26%) in Molise.

A total of 205 samples (44.2%) were taken from supermarkets and 187 (37.1%) from butchers, 259 (66%) from loose products and 133 (34%) from packaged products.

Sectioned products accounted for 95.1% (373 samples) of the total samples tested.

A sampling sheet reporting the following information was completed for each sample: shop details (name, type and address), producer and product presentation.

Each sample was taken from a different shop and was selected randomly from the available poultry meat pieces. Products were sealed in a sterile food bag, labelled and stored at 4°C until delivery to the laboratory where the accuracy of sampling sheet information and the transportation temperature were checked.

A total of 25 g of product were taken from each sample and homogenised in a stomacher bag containing 225 ml of Preston broth (Biolife, Italy and Oxoid, UK). Thermotolerant (able to grow at 42°C) *Campylobacter* detection was performed using the ISO 10272:1995 method (22) and enumerated according to the most probable number (MPN) method described in the *Bacteriological analytical manual* (17), using three dilution series.

Samples were incubated for 24 h at 42°C, in microaerophilic conditions, using commercial kits (CAMPYgen, Oxoid, UK). After incubation, three selective media were inoculated, namely: Karmali (Biolife, Italy) and Skirrow (Oxoid, UK), specified in ISO 10272:95 (22) and modified CCDA (Oxoid, UK), introduced by the proposed amendment of the same method (24). After microaerophilic incubation for 72 h at 42°C, the colonies that corresponded morphologically to *Campylobacter* spp. were selected and purified for confirmation tests. Biochemical tests as specified by ISO 10272:1995 (22) were used for complete identification.

Campylobacter spp. contamination levels were calculated from the positive dilutions for typical colonies isolation and interpreted using the appropriate MPN tables (17).

A comparison between levels of loose and packaged products and between small- and large-scale distribution channels was made using a χ^2 test (34).

Results

Thermotolerant *Campylobacter* was isolated in 160 samples (40.8%) using the ISO method (42°C for incubation). Contamination by product (type and description) and distribution channel is presented in Table I.

No statistically significant difference was seen in contamination levels of loose and packaged products (χ^2=0.25; P=0.6198) nor between small- and large-scale distribution channels (χ^2=2.27; P=0.1317).

Contaminated samples generally contained a low concentration of *Campylobacter* spp. (Fig. 1), with 130 of 160 positive samples showing levels of 0.3 to 110 MPN/g. The remaining 30 samples found positive on qualitative testing were found negative by the MPN method, with concentrations of 0.04-0.3 MPN/g (Fig. 1).

Campylobacter jejuni was isolated in 131 of 160 positive samples (81.9%) and *C. coli* in 52 samples (32.5%). Other species were identified in 17 samples (10.6%) (Table II).

Overall, 265 strains of *Campylobacter* spp. were isolated. They were identified using biochemical methods, as follows: 181 (68.3%) were classified as *C. jejuni*, 57 (21.5%) as *C. coli* species and 27 (10.2%) as *Campylobacter* spp. (*C. upsaliensis* and *C. mucosalis*) (Table II). Three strains were not identified. More than one species of *Campylobacter* was observed in 23.1% of positive samples (Table III).

Discussion

The results of the survey confirm a high prevalence of contamination with thermo-

Vol. 43 (1), Vet Ital

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Table I
Percentage of poultry meat samples that gave positive results to thermotolerant *Campylobacter* in relation to type of product and distribution channel

Type of product	Presentation of product	Samples from large-scale retail stores			Samples from small-scale retail stores			Total		
		Tested	Positive (%)	Confidence limit (95%)	Tested	Positive (%)	Confidence limit (95%)	Tested	Positive (%)	Confidence limit (95%)
Whole	Loose	3	3 (100.0%)	(39.76-99.37%)	7	3 (42.9%)	(15.7-75.51%)	10	6 (60.0%)	(30.79-83.25%)
	Pre-packaged	9	7 (77.8%)	(44.39-93.33%)	0	0	(0-97.5%)	9	7 (0.0%)	(44.39-93.33%)
	Sub-total	12	10 (83.3%)	(54.55-94.96%)	7	3 (42.9%)	(15.7-75.51%)	19	13 (68.4%)	(45.72-84.61%)
Sectioned	Loose	80	39 (48.8%)	(38.08-59.53%)	169	63 (37.3%)	(30.34-44.79%)	249	102 (41.0%)	(35.04-47.17%)
	Pre-packaged	113	42 (37.2%)	(28.81-46.39%)	11	3 (27.3%)	(9.92-57.19%)	124	45 (36.3%)	(28.35-45.07%)
	Sub-total	193	81 (42.0%)	(35.23-49.03%)	180	66 (36.7%)	(29.97-43.93%)	373	147 (39.4%)	(34.58-44.46%)
Total		205	91 (44.4%)	(37.75-51.24%)	187	69 (36.9%)	(30.31-44.02%)	392	160 (40.8%)	(36.06-45.75%)

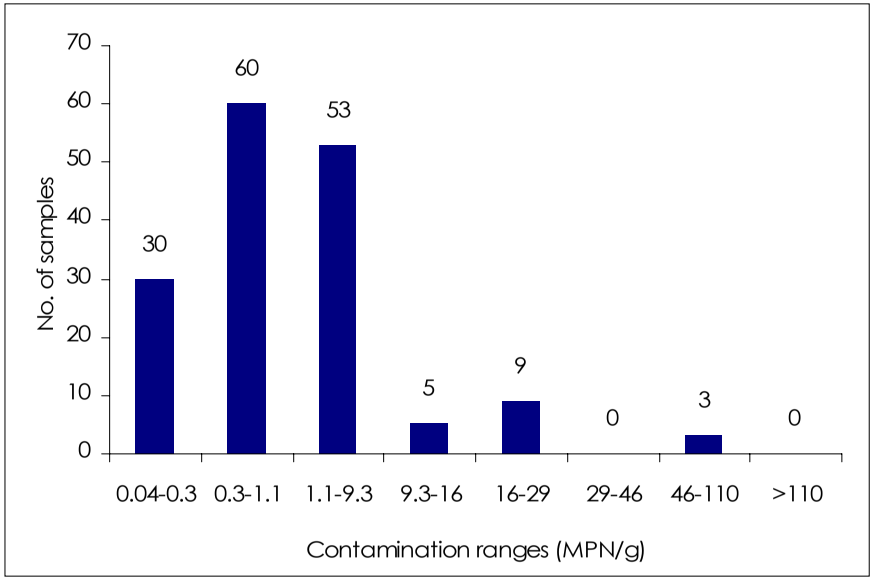

Figure 1
Contamination ranges of chicken samples with different *Campylobacter* spp.

Table II
Identified species of thermotolerant *Campylobacter*

Species	No. of strains isolated (%)	No. of samples (%)
Campylobacter jejuni	181 (68.3%)	131 (81.9%)
Campylobacter coli	57 (21.5%)	52 (32.5%)
Other species	27 (10.2%)	17 (10.6%)

Table III
Number of identified thermotolerant *Campylobacter* species in individual samples

No. of species per sample	*Campylobacter* species	No. of samples	Samples (%)
1	*C. coli* *C. jejuni* ssp. *doylei* *C. jejuni* ssp. *jejuni* *C. upsaliensis* *Campylobacter* sp.	103	64.4
2	*C. jejuni* ssp. *doylei*, *C. coli* *C. jejuni* ssp. *jejuni*, *C. upsaliensis* *C. jejuni* ssp. *doylei*, *C. jejuni* ssp. *jejuni* *C. jejuni* ssp. *doylei*, *C. upsaliensis* *C. jejuni* ssp. *jejuni*, *C. coli*	47	29.4
3	*C. jejuni* ssp. *jejuni*, *C. coli*, *C. upsaliensis* *C. jejuni* ssp. *doylei*, *C. jejuni* ssp. *jejuni*, *C. upsaliensis* *C. jejuni* ssp. *doylei*, *C. jejuni* ssp. *jejuni*, *C. coli*	9	5.6
4	*C. jejuni* ssp. *doylei*, *C. jejuni* ssp. *jejuni*, *C. coli*, *Campylobacter* spp.	1	0.6

tolerant *Campylobacter* in poultry meat in small- and large-scale retail stores in the Abruzzo and Molise area. Whole and sectioned meat samples were chosen as being the most widely consumed and sold products. The level of contamination with *Campylobacter* observed in this study (40.8%) falls between the values observed by other authors (9, 25, 27, 33, 35, 36). No statistically significant differences in contamination levels were found between the two distribution channels or product type (packaged or loose), in contrast with the hypothesis of Zhao *et al.* (37). According to these authors the prevalence of product contamination is influenced by a series of factors: time of year when sampling is performed, product storage type, sampling time, product batch. The lack of such differences in our survey may be due to the brief sampling duration or the number of samples.

The literature reports variable contamination levels in poultry products, from several hundred to millions of *Campylobacter* microorganisms in a single gram of product (12). It should be noted that these results were obtained using different sampling criteria and analytical procedures that seriously affect the overall sensitivity of the survey methods, thus compromising any attempt at a comparison of data (15, 25). This is particularly valid for quantitative determinations, as demonstrated by inter-laboratory testing in the United Kingdom (18).

The most common species found in chicken meat was *C. jejuni* (81.9% of analysed samples, accounting for 68.3% of isolated strains) (Table II). Other surveys report isolations of 77.3% (26), 74% (18) and 68% (27). Furthermore, as also reported by other authors (26, 30), multiple *Campylobacter* species may be found in the same sample. Molecular biology studies of *Campylobacter* isolated from patients suffering from gastroenteritis confirm the contemporary presence of different species in 5-10% of patients (26).

In the light of our results, it is interesting to observe that in 2002 no further cases of gastroenteritis caused by *Campylobacter* were reported in Abruzzo and Molise, while only five cases were notified (15) in other regions of Italy in the same year. In the same period in other European countries, the incidence per 100 000 people was between 2.3 (France) and 101.1 (Scotland) (15). The absence of official data in Italy is due to the lack of specific surveillance, as implemented in other European countries and the lack of specific registration of *Campylobacter* infection, which is included in Class IV (infections, toxic infections and infestations of animal origin) of the Ministerial Decree of 15 December 1990 (4)

and thus is not discriminated from other foodborne infections.

In Denmark, where per capita consumption of chicken meat in 1990 was 19 kg (35), comparable to that recorded in Italy (18.5 kg) (35) the same year, 4 385 cases of human *Campylobacter* infection were reported, equivalent to 88.7 cases per 100 000 people (15).

Applying the Danish incidence to the Italian population in 2002, a total of 50 844 cases should have been recorded. If we consider that 20-40% of all sporadic cases involve the consumption of poultry meat (28, 35), in the same period, there should have been between 10 169 and 20 337 cases due to poultry consumption alone.

These estimates are obviously approximate and do not take into consideration any differences in dietary habits between Italy and Denmark or other risk factors responsible for human infection. However, it is also true that, given the similar level of poultry contamination in other European countries and considering similar levels consumption, the number of human cases which should be expected in Italy is higher than that currently recorded.

Recent European legislation in the area of zoonoses (13, 14) requires the application of national notification systems for human infectious diseases without delay. Awareness of the real incidence of infection is essential to ensure the success of precautionary measures applied to reduce the number of cases of infection.

Fewer infections in livestock and the application of appropriate hygiene measures to control diffusion and cross-contamination in processing phases up to and including retail sale are the main goals of actions to control the level and frequency of meat contamination.

It is also necessary to implement national surveillance systems to measure both the prevalence and level of product contamination and to estimate consumer risk. Finally, it would be desirable to set up coordinated survey systems that use genotyping techniques, such as pulsed field gel electrophoresis (PFGE) and multi locus sequence typing (MLST), to assist in the interpretation of the epidemiological data and the delivery of information on the presence of persistent strains and niches throughout the production chain (12).

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