

Occurrence of *Campylobacter* spp. in poultry meat at retail and processing plants' levels in Central Italy

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Abstract

Human campylobacteriosis remains the most commonly reported gastrointestinal disease in Europe and *Campylobacter* (*C.*) *jejuni* and *C. coli* are the two species most frequently involved in such foodborne disease. Based on the sampling plan established in the region of Lazio (Central Italy) the aim of our work was to investigate the occurrence of *Campylobacter* spp. in poultry meat preparations collected by the local veterinary authority at retail shops and processing plants. We also observed whether various factors such as animal species or type of product affected the isolation rate. Occurrence was significantly lower than previous surveys (12/209, 5.7%) and chicken meat was more contaminated than turkey meat.

Introduction

Campylobacter spp. are Gram negative, microaerophilic, curved or spiral rods in the family *Campylobacteriaceae*. The genus *Campylobacter* includes more than 20 species and subspecies isolated from humans and/or animals and some of these cause a variety of infections in humans and animals as well. In particular, *C. jejuni* and *C. coli* are the pathogens most commonly involved in foodborne diseases in Europe and the USA (EFSA, 2015; Spickler, 2013; Humphrey *et al.*, 2007).

Campylobacter spp. have been found in the intestines, oral cavity, and reproductive organs of many warm blooded animals and humans; usually infected animals are asymptomatic and since these microorganisms are spread in the environment through feces, they can also be found in soil and water (Spickler, 2013; Schaffter *et al.*, 2004). Human infection may occur by direct contact with infected animals or by consumption of contaminated unpasteurized milk, dairy products, untreated water and

raw meat. Wild and domestic birds have been considered as one of the most important reservoirs of foodborne infection for humans. In fact, *Campylobacter jejuni* and *coli* are more frequently isolated from poultry than other species and the consumption of contaminated poultry meat is the leading cause of domestically acquired foodborne illnesses in humans (Humphrey *et al.*, 2007; EFSA, 2015).

Moreover, human campylobacteriosis remains the most commonly reported gastrointestinal disease in Europe (EFSA, 2015) and in most industrialized countries in the world (Hermans *et al.*, 2012; Wilson *et al.*, 2008) and broiler meat is considered to be the main source of infection (EFSA, 2015). *C. jejuni* and occasionally *C. coli* cause enteritis in humans; the infectious dose for *Campylobacter* is low (few hundred cells) and the incubation period ranges from one to ten days (Humphrey *et al.*, 2007). The symptoms may include watery or sticky diarrhea, fever, nausea, vomiting, abdominal pain, headache and muscle pain. In some cases, *Campylobacter* infection may trigger severe complications, including reactive arthritis and Guillain-Barré syndrome (acute, progressing paralysis) (Spickler, 2013).

According to a review, which included several worldwide surveys (Suzuki and Yamamoto, 2009), the mean prevalence of *Campylobacter* spp. in retail poultry meat in Europe was 53.3%. However, prevalence between countries varied greatly (from 8.1 to 80.0%). Regarding Italy, only few studies have been carried out at retail level (Nobile *et al.*, 2013; Sammarco *et al.*, 2010; Parisi *et al.*, 2007; Pezzotti *et al.*, 2003) and prevalence ranged from 20.7 to 81.3%. Such studies concerned only some regions, therefore data is missing from many Italian territories.

On the other hand, to our knowledge, the prevalence of *Campylobacter* spp. in poultry meat collected at the processing plant level in Italy has not yet been reported in literature while only few studies have been carried out elsewhere in the European Union; two separate studies conducted in Belgium and Germany reported, respectively, a prevalence of 22.1% in broiler fillets and up to 79.0% in different turkey meat preparations (Ghafir *et al.*, 2007; Hamedy *et al.*, 2007).

Due to the relevance of *Campylobacter* as an agent of foodborne disease, the veterinary authority of the region of Lazio established, since 2011, a specific sampling/control plan regarding poultry meat at processing plants and retail. Therefore, the purpose of our study was to report the occurrence of *Campylobacter* spp. in poultry meat preparations collected from retail shops and processing plants in central Italy. We also observed whether various factors such as animal species or type of product affected the isolation rate.

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Materials and Methods

Between 2011 and 2014, a total of 209 poultry meat samples (162 chicken, 34 turkey and 13 mixed) were collected by the local Veterinary authorities at retail shops (156) and processing plants (53) spread across the Italian region of Lazio. The proportional distribution of samples among the local veterinary authorities was carried out by taking into consideration the number of existing retail shops/processing plants within the relative territories. The plan established the sampling of only fresh meat preparations (no frozen products) irrespective of the packaging status. Samples were refrigerated and delivered within 24 hours to the laboratory. All samples were prepared according to the specific rules of ISO 6887-2 (ISO, 2003) and isolation of *Campylobacter* spp. was performed in accordance with ISO 10272-1 (ISO, 2006).

Briefly, the detection method consisted of inoculating 25 g of the sample into 225 ml of a selective pre-enrichment medium (Bolton Broth) and incubating in a microaerophilic atmosphere (Campygen; Oxoid, Basingstoke, UK) at 37°C for 4-6 h and then at 41.5°C for 44±4 h. After enrichment, the Bolton Broth was plated onto two different selective media (Modified Charcoal Cefoperazone Deoxycholate Agar and *Campylobacter* Selective Medium, Skirrow) and incubated for 48 h at 41.5°C in a microaerophilic atmosphere. After incubation, between one to five

typical colonies were plated onto Columbia Blood Agar (ASC; Biolife, Milan, Italy) and incubated for 48 h at $41.5 \pm 1^\circ\text{C}$ in a microaerophilic atmosphere. *Campylobacter* cultures were then confirmed by microscopic observation to examine the morphology and motility of isolates followed by the oxidase test, the mobility test, and a double growth test at $25 \pm 1^\circ\text{C}$ in a microaerophilic atmosphere and at $41.5 \pm 1^\circ\text{C}$ in aerobiosis.

Confirmation of isolates and identification of the relative *Campylobacter* species was performed using two different PCR qualitative assays; the first, used as a screening method, is a commercial real time PCR kit method (Adiafood Detection System *Campylobacter*; bioMérieux, Marcy l'Etoile, France) that enables the amplification of the target DNA using specific primers and molecular beacons designed to detect common sequences of the three thermotolerant *Campylobacter* species: *C. coli*, *C. jejuni*, and *C. lari*. The second, which is a multiplex PCR, was performed to distinguish between *C. jejuni* and *C. coli* using two different pairs of primers (*C. jejuni*-specific *hipO* gene sequence of 735 bp (Linton *et al.*, 1997) and a 500 bp fragment of a *C. coli* putative aspartokinase gene (Linton *et al.*, 1997).

The χ^2 test was performed to evaluate the presence of a significant association ($P < 0.05$) between prevalence and factors such as sampling stage, type of product (minced meat preparations versus meat preparations) or packaging status.

Results

Campylobacter spp. was isolated from twelve poultry meat samples (12/209, 5.7%). Table 1 shows the number of positive poultry meat samples classified by type of product. *Campylobacter* was detected in eleven chicken meat samples (11/162, 6.8%) and isolates were identified as follows: 7 were *C. coli*, 3 were *C. jejuni* species and one isolate was different from *coli/jejuni* species. None of the turkey meat preparations resulted positive (0/34) while two different strains of *Campylobacter* (*jejuni* and *coli*) were isolated from one mixed meat preparation (1/13, 7.7%).

In consideration of the sampling stage, the occurrence was 6.4% for samples collected at retail (10/156) and 3.8% for those collected at the processing plants (2/53). The occurrence of the pathogen was 10.7% (6/50) in unpackaged products and 2.7% (3/108) in packaged products. The information regarding the packaging status of 39 meat preparations was not provided by the local veterinary authorities (3 positive samples, 7.1%).

Statistical analysis did not show any association between occurrence and the factors we considered even though the packaging status was nearly significant ($P = 0.053$).

Discussion

Although surveys from international literature about *Campylobacter* in poultry meat can differ in sampling design and testing methods, a comparison with the results of the present study can still be made with caution. Surprisingly, the occurrence is significantly lower (5.7%) if compared with the other Italian surveys (Nobile *et al.*, 2013; Sammarco *et al.*, 2010; Parisi *et al.*, 2007; Pezzotti *et al.*, 2003); in general, it was also lower than all the prevalence studies regarding poultry meats and by-products performed in other countries (Suzuki and Yamamoto, 2009). The reason beyond this low rate might be explained, as assumed by Nobile *et al.* (2013), by the fact that in recent years a higher level of attention regarding slaughter hygiene has been applied in order to reduce the risk of contamination. Furthermore, the above-mentioned studies concerned samples collected at retail whereas in this study samples were also gathered at the processing plant level where the occurrence of *Campylobacter* is usually lower (Padungtod and Kaneene, 2005; EFSA, 2015).

All the isolates except one proved to belong to *C. jejuni* and *coli* species (11/12). Similar high proportion has been also observed in several surveys (EFSA, 2015; Suzuki and Yamamoto, 2009) and underlines the relevant impact of contaminated poultry meat on public health in consideration of the major zoonotic role of such species.

The ratio between the two species has been strongly in favor of *C. coli*; a similar result has been reported by Nobile *et al.* (2013), while the studies from Pezzotti *et al.* (2003) and Sammarco *et al.* (2010) have reported higher *jejuni* isolates. In general, the international literature supports such variability of the ratio between those two species in poultry meat (Suzuki and Yamamoto, 2009).

Chicken meat preparations turned out to be

more contaminated than turkey meat preparations (none of the samples resulted positive) and preparations made with mixed meat (*i.e.* chicken and turkey meat). This is in line with a recent EU report 2013 (EFSA, 2015) where the occurrence of *Campylobacter* was higher in chicken meat compared to other poultry species (mainly turkey).

Higher occurrence has been detected in samples collected at retail compared to those gathered at processing plants. Similar results have been reported in other studies and suggest that such difference might be due to cross-contamination as a result of greater handling of the products at retail (Padungtod and Kaneene, 2005; Nobile *et al.*, 2013). Cross-contamination might also explain the higher prevalence rate that has been detected between unpackaged and packaged poultry meat products.

Minced meat preparations resulted less contaminated than other meat preparations in agreement with other studies and reports (Suzuki and Yamamoto, 2009; EFSA, 2015). On the other hand, chicken thighs were the most contaminated (6/51) probably because such cuts are most at risk in view of the anatomical proximity to the final part of the digestive tract.

Conclusions

In conclusion, considering the remarkable variability of prevalence between the Italian regions, a national-scale survey on *Campylobacter* spp. in poultry meat based on the same sampling design and testing method should be established in order to obtain more accurate data. In any case, the detection of *Campylobacter* spp. in poultry meat should remain a priority for the veterinary authorities in view of the facts that almost all isolates belonged to the two species most frequently

Table 1. Distribution of poultry meat samples according to type of product.

| Matrix information | Sample type | Total units tested (n) | Total units positive (n) |
|--------------------------|----------------|------------------------|--------------------------|
| Whole carcasses | | 9 | 0 |
| Minced meat preparations | Hamburgers | 27 | 1 |
| | Fresh sausages | 15 | 0 |
| | Other | 24 | 0 |
| | Total | 66 | 1 |
| Meat preparations | Wings | 9 | 0 |
| | Thighs | 51 | 6 |
| | Breasts | 42 | 2 |
| | Other | 32 | 3 |
| | Total | 134 | 11 |
| Total | | 209 | 12 |

associated with human campylobacteriosis and that a high number of reported cases still occur each year in Europe.

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