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Prevalence and Characterization of *Campylobacter* spp. in Poultry Meat: Results of a-year Surveillance in the North-West of Italy

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Abstract

Campylobacter spp. represents the most common cause of foodborne illness occurring in Europe. Regarding to food safety, the contaminations mainly concerns raw milk and poultry meat. In this case, consumers can get infected through the consumption of undercooked poultry meat or through cross-contamination in ready-to-eat-foods preparation. In the last decade, *Campylobacter* spp. diffusion and characterization have been defined in many European countries. No official data are currently available about the contamination of poultry meat at retail in North-west Italy. The aim of this study was to investigate the occurrence and seasonality of *Campylobacter* spp. in samples of poultry raw meat sold in retails of the Piedmont Region (north of Italy). A panel of 500 chicken meat samples have been processed to verify the presence of *Campylobacter* spp. The samples were collected from randomly selected retails. Analytical tests determined *Campylobacter* spp. positivity in 32 (6%) of the total analysed samples. The most frequent isolates were *Campylobacter coli* (66%), *Campylobacter jejuni* (22%), mixed *C. coli* and *C. jejuni* (3%). The remaining 9% has not been identified. Our findings revealed a higher occurrence of *Campylobacter* spp. in chicken thighs than in chicken breasts particularly during summer season. Despite the relative low prevalence (6%) in the Piedmont Region, this study highlights the critical role of raw poultry meat in spreading *Campylobacter* spp. strains. "One Health" approaches represent a strategy to control the *Campylobacter* spp. Further programmes, policies, legislations and researches will contribute to control contaminations at primary production level and retail Stores.

Keywords: *Campylobacter spp; Poultry Meat; Retail Shop*

Introduction

The bacteria belonging to the genus *Campylobacter* are primary pathogens causing human Campylobacteriosis. Referring to EU countries, *C.* spp. is one of the most reported gastrointestinal pathogen in humans, representing almost 70% of all recorded cases since 2005 [1]. In 2017, a total number of 246.158 confirmed human cases occurred in the European Union Countries, with notification rate of 64.8/100,000 inhabitants, representing the third cause of mortality from foodborne pathogens [1]. In respect with human cases, epidemiological analysis demonstrated a seasonality of onset [1] and a higher isolation rates of *C.* spp. from chickens, predominantly in the summer months [2]. Regarding the previous years, in 2017 EFSA reports the highest country-specific notification in Czech Republic (230.0/100,000), Slovakia (127.8/100,000), Sweden (106.1/100,000) and Luxembourg (103.8/100,000). These microorganisms are Gram-negative, mainly presenting a microaerophilic metabolism. They also are characterized by s-shape morphology with unipolar or

bipolar flagella to allow motility. Normally, the infection causes an inflammatory response characterized by bloody diarrhea or dysentery syndrome, mostly including cramps, fever, pain, nausea, and vomiting. In some cases, the pathology could degenerate in complications such as arthritis, endocarditis and Guillain-Barré syndrome or its variant the Miller-Fisher syndrome [3]. The most important species associated with human infection in Europe are *C. jejuni,* followed by *C. coli* and *C. lari* [1]. The origin of the infection mainly concerns not only foods as raw milk [4], poultry meat [5], contaminated water sources, but also international travellers [6] and infected pets [7] could also be the source of infections. Wild birds may play a role in the diffusion of *C. spp*. in areas adjacent to farms [8]. Therefore, the diffusion of the microorganism is mainly related to the consumption of contaminated foods such as undercooked poultry meat, raw milk, and cross-contaminated ready-to-eat foods. *C. spp*. diffusion can easily occur through cross-contamination during foods preparation [9]. A qualitative cross-contamination study demonstrated that *C. jejuni* contaminations may readily occur among raw chicken products, hands, cutting boards, plates and others kitchen utensils [10]. Accordingly, Campylobacteriosis is generally considered as a foodborne illness. Referring to fresh meat, six European Member State, in 2017, reported monitoring data on *C.* spp. highlighting an occurrence rate ranging from 37.4% to 31.5% in broilers and turkeys, respectively. In addition to the few data available, the sampling and reporting rules are not harmonised, precluding good trend analyses and trend watching [1]. The primary infections caused by *C.* spp. in Europe (excluding strong evidence waterborne outbreaks) during 2017 were associated with contaminated milk (54.5%), broiler meat and their secondary products (24.2%), mixed or unspecified poultry meat and their products (6.1%), mixed red meat (6.1%), dairy products (other than cheeses) (6.1%) and meat products (3%) [1]. From 1th January 2018, the Regulation (EU) 2017/1495 has amended the Reg. 2073/2005, to reduce the number of human Campylobacteriosis cases attributable to the consumption of poultry meat, introducing a process hygiene criterion for *C.* spp. on broiler carcases, establishing also three class sampling plan. Specifically, as provided for in Regulation, the enumeration of *C*. spp. shall be carried out on fifty sample units during the slaughtering process after chilling. Since 2020, referring to broilers meat, the EU Regulation will provide for a progressive decrease in tolerance (c) of positive sample units (< 1000 cfu/g) from a c = 15 to a c = 10 established for 2025 [11]. In Piedmont Region (Northern Italy), the last study of *C.* spp. diffusion dates back to 2001 regarding the contamination of raw milk [12]; no data are reported about *C.* spp. diffusion on poultry meat and secondary products. The aim of this study is to investigate the occurrence and seasonality, of *C.* spp. in different chicken products at retail stores of the Piedmont Region.

Material and Methods

A total of 500 samples of pre-packaged chicken meat were collected monthly between July 2015 and June 2016 from different butcher's shop and markets of the Piedmont Region in the north-west of Italy. Samples included breast and thighs equally shared, were collected from randomly selected supermarkets, based on the number of inhabitants per province. Pre-packaged chicken meat was not stored in a modified atmosphere (MAP). Meat samples were immediately transported to the laboratory on ice, refrigerated and processed within 24 hours from collection. The detection and the enumeration of *C.* spp. were performed according to ISO 10272:2006 part 1 and 2 (accredited ISO17025 by Accredia).

Data were analysed using SAS® ver. 9.2 (SAS Institute Inc., Cary, NC, USA). Descriptive analysis was performed to determine the occurrence of pathogens. The χ^2 test was applied to test the associations between the occurrence of pathogens and the season. The strength of the association has been assessed by odds ratio calculation. The significance of the predictors in the model was set at $\alpha = 0.05$ and tested using Wald's chi square.

Results and Discussion

In Italy, the real impact of Campylobacteriosis has not been established properly due to the lack of an active surveillance plan on the prevalence of clinical cases. Furthermore, this is accompanied by limited data on the distribution of *C.* spp. in foods as for example in poultry meat. Our study represents a new monitoring contribution about the prevalence of *C.* spp. in poultry raw meat at retail. Starting from 2002 a decreasing trend of the occurrence of *C.* spp. on poultry meat seems to be noticeable in Italy: in 2002, the registered occurrence was

81% for north-eastern Italy [13], in 2006, an occurrence of 73% was observed for south-eastern Italy [14] and in 2009, the measurement was 51% the central Italy [15]. The prevalence of *C.* spp. on poultry meat in south-western Italy was registered at 20.7% [16] during 2013. In our study, *C.* spp. was detected on 32 (6%) out of 500 samples (Figure 1). Although these prevalence data in the various Italian regions have not been harmonized, the *C.* spp. trend decrement may have been determined by an increase in hygiene and safety measures during the production chain applied in the last years. Milk and broiler meat represent the main source of *C.* spp. in respect with human infections [1]. Surveillances carried out in Spain, Italy and France, demonstrated that poultry meat carcasses and legs with skin are more contaminated than fillets without skin [17-19]. Moreover, as reported in previous studies, chicken thighs resulted more contaminated than breast products. Specifically, the microorganism was present in 12 chicken breasts samples (38%) and 20 thighs (62%) (Figure 2). Higher population rates of *C.* spp. on samples with skin were found also by other author; they demonstrate the peculiar attachment properties of the bacteria on the follicular structure of the skin. Furthermore, chicken skin has a role in protecting and supporting the survival of *C.* spp., thus representing a possible source of infection [20]. In accordance, our study revealed a higher occurrence of *C.* spp. contaminations on chicken thighs (with skin) than skinless breast samples ($p < 0.05$), probably because thighs are sold with skin. In our research, the identified species were *C. coli* and *C. jejuni; C. coli* was detected in 21 (66%) samples (8 breasts; 13 thighs), while *C. jejuni* in 7 (22%, 1 breast and 6 thighs) (Figure 3). Positivity for both species has been observed in one chicken breast (3%) (Figure 3). For 3 (9%) of the breast samples, the biochemical identification of *C.* spp. was uncertain (Figure 3). Therefore, like other studies conducted in central Italy [21] and southern Italy [14] the present study demonstrated a predominance of *C. coli* versus *C. jejuni* species. In survey conducted in Poland, *C. coli* showed a prevalence of 75.5% on *C.* spp. positive samples [22]. Moreover, in Greece, *C. coli* demonstrated to be the predominant species in chicken meat, as well as in lamb and goat carcasses [23]. Unlikely, previous studies reported high predominance of *C. jejuni* in broilers sold in Europe with different prevalence among countries [24]. There are many risk factors may increase the colonization of broiler flock by *C.* spp. [25]. During food manufacturing, cross contaminations could play an important role in the diffusion of *C.* spp. Therefore, the management and the application of HACCP manuals, (in particular Good Manufacturing Practices GMP, and Good Hygiene Practice GHP) may efficiently overcome these risk factors. In this respect, food operators of poultry slaughtering companies are subject to constant updating courses on HACCP procedures (an upgrade session every two years). Furthermore, food chain companies (under authorization of Food Safety Competent Authority as provided for in Regulation 853/2004) apply self-regulation plans focusing on sanitization procedures during animals slaughtering (e.g. carcasses chilling). In addition to that, slaughterhouses undergo to periodic risk assessment controls by Food Safety Competent Authority.

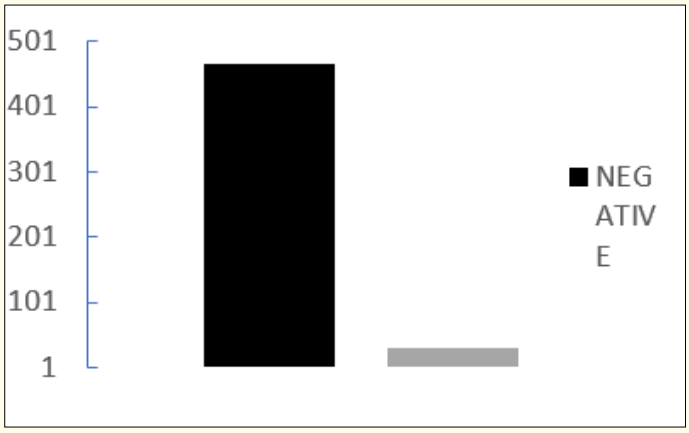

Figure 1: *Histogram representing the detection of C. spp on poultry meat at retail stores of the Piedmont Region (from July 2015 to June 2016). Y axis indicates the total number of analysed samples. X axis shows the whole proportion of positive and negative samples. Positive samples are in grey, negative samples are in black.*

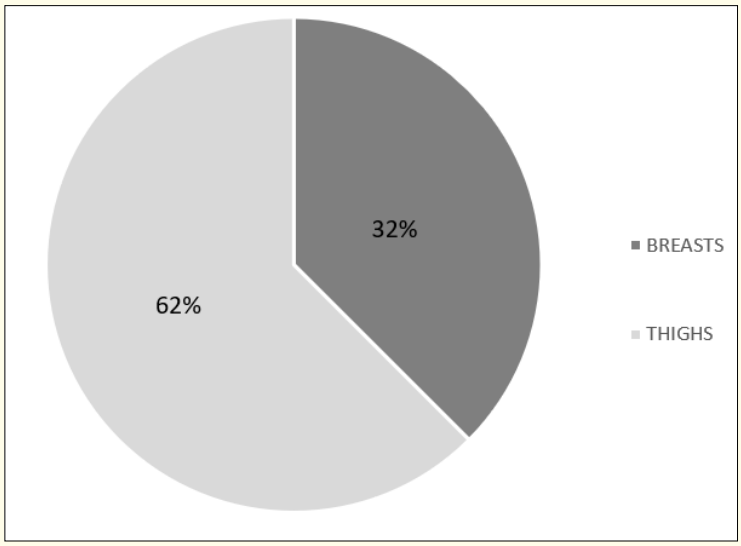

Figure 2: *Pie chart showing distribution of positivity relating to C. spp. on chicken breasts and thighs. Light grey area corresponds to breast positive samples. Dark grey area represents thigh positive samples. Percentages are reported in the graphic.*

The statistical analysis on seasonality occurrence of *C.* spp. reveals a higher presence of contaminated samples during the summer and autumn versus winter and spring ($p < 0.05$) (Figure 4). Odds ratio indicates9x higher probability to encounter a contaminated poultry meat sample in summer than in winter.

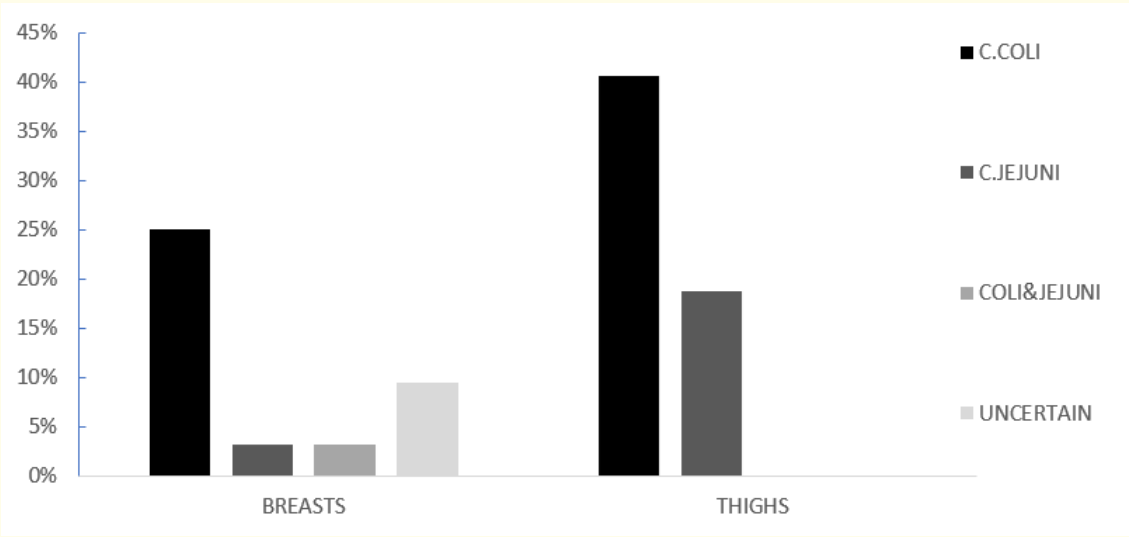

Figure 3: *Histograms representing the distribution of C. spp species on breasts and thighs of analysed poultry meat. Y axis indicates the percentages of positive samples. X axis shows the breast and thigh histograms related to the presence of C. coli, C. jejuni, both species, and not identified species as explained in the legend.*

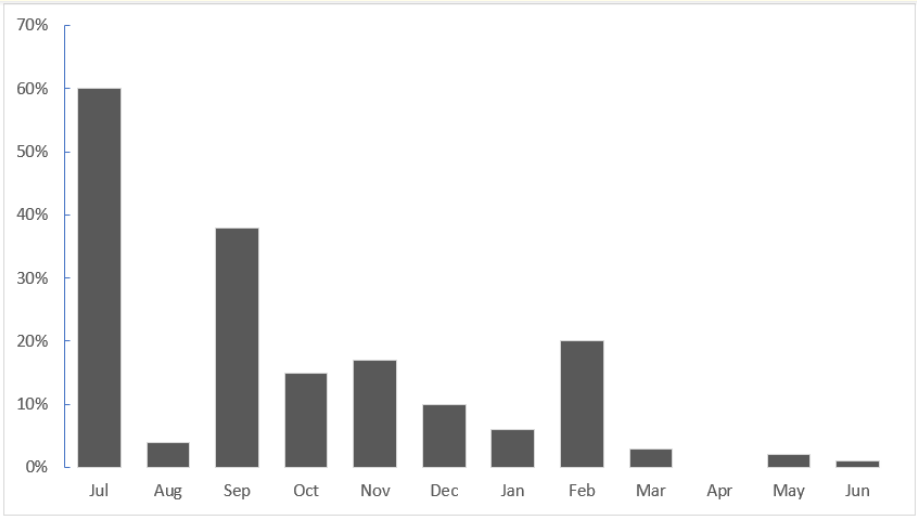

Figure 4: *Seasonal Variation of C. spp. Graphic representing positivity trend of C. spp. on poultry meat samples from July 2015 to May 2016. X axis reports the months of one-year surveillance. Y axis indicates the percentages related to the positive samples. Histograms represent positive samples related to a specific month.*

These results are comparable with those published in the *EFSA* 2017 reports. Accordingly, Garcia., *et al.* showed a significant increase of human Campylobacteriosis cases during summer [17]. We speculate an involvement of the temperature variability among the seasons as well as the variation of climatic conditions in respect with occurrence of *C.* spp. contamination on poultry meat. As reported in previous studies [16], the collection of samples at retail shops increases variability of meat origin and allows to obtain an overview on consumer exposure to the bacterial agent. Regarding to the Piedmont Region, the low occurrence of *C*. spp species showed in this study, demonstrates that chicken meat at retail, is poorly contaminated thus having a low impact on public health.

Conclusion

In the last decade, numerous studies in E.U. described the occurrence of *C.* spp. contamination in foods. In respect with poultry meat and its secondary products, the trend of the *C.* spp. occurrence in E.U. needs to be properly defined, mainly through harmonizing reports from various monitoring data. Specifically, this procedure may help the authorities to understand trends and sources of *C.* spp. along the food chain, and consequently implement specific prevention measures. This study provide key informations, which should be useful for Food Safety Competent Authority and food operators in the control of Campylobacter contaminations during poultry meat based products preparation, with respect to good manufacture procedures and risk based assessment.

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