http://dx.doi.org/10.1016/j.fm.2016.10.028

Prevalence and quantification of thermophilic *Campylobacter* spp. In Italian retail poultry meat: Analysis of influencing factors

Simone Stella [a, *], Gabriella Soncini [a], Graziella Ziino [b], Antonio Panebianco [b], Francesca Pedonese [c], Roberta Nuvoloni [c], Elisabetta Di Giannatale [d],Giampaolo Colavita [e], Leonardo Alberghini [f], Valerio Giaccone [f]

[a] Department of Health, Animal Science and Food Safety, Università degli Studi di Milano, via Celoria 10, IT-20133, Milano, Italy

[b] Department of Veterinary Sciences, Università degli Studi di Messina, Polo Universitario dell'Annunziata, Viale dell'Annunziata, IT-98168, Messina, Italy

[c] Department of Veterinary Sciences, Università di Pisa, viale delle Piagge 2, IT-56124, Pisa, Italy

[d] Italian National Reference Laboratory of Campylobacter, Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Via Campo Boario,
IT-64100, Teramo, Italy

[e] Department of Medicine and Health Science, Università degli Studi del Molise, Via de Sanctis s.n.c., IT-86100, Campobasso, Italy

[f] Department of Animal Medicine, Production and Health, Università degli Studi di Padova, Agripolis, Viale dell'Università16, IT 35020, Legnaro, PD, Italy

* Corresponding author.
E-mail addresses: simone.stella@unimi.it (S. Stella), gziino@unime.it (G. Ziino), apanebianco@unime.it (A. Panebianco), francesca.pedonese@unipi.it (F. Pedonese), roberta.nuvoloni@unipi.it (R. Nuvoloni), e.digiannatale@izs.it (E. Di Giannatale), colavita@unimol.it (G. Colavita), leonardo.alberghini@unipd.it (L. Alberghini), valerio.giaccone@unipd.it (V. Giaccone).

ABSTRACT

Retail poultry meat is a crucial vehicle for consumers' exposure to Campylobacters, but no official controls are currently applied in Italy. The aim of this study was the evaluation of *Campylobacter* contamination of a wide range of poultry meats marketed in Italy. N. 472 chicken and turkey meat

samples (sectioned meats, offal, meat preparations and products) were taken from slaughterhouses, deboning plants and different retailers and submitted to detection/enumeration of *Campylobacter* spp. The isolates were identified by phenotypic and biomolecular techniques. *Campylobacter* spp. was detected in 34.1% of the samples, with general low counts. Higher values were observed in offal (especially liver) and sectioned meats, with significantly higher rates in skin-on samples (86.8% vs 32.7%). Minced meat preparations showed lower prevalence (22.4% vs 58.3%) and counts than whole pieces. Decreasing rates were observed among slaughterhouses (80%), deboning plants (49%), butcher's shops (37%) and large scale retailers (25%). Sectioned chicken meats were significantly more contaminated than turkey meats.

Almost all the isolates were identified as *C. jejuni* or *C. coli*, with similar prevalences (18.4% and 20.5%, respectively); *C. jejuni* was predominant only in samples from slaughterhouses/deboning plants. For setting future control programs, meat typology should be considered the main critical factor.

Keywords: *Campylobacter*, Retail, Poultry meat, Italian market.

1. Introduction

Campylobacteriosis is by far the most common foodborne infection in the European Union, with more than 200.000 confirmed human cases/year (EFSA-ECDC, 2015). Data from Italy show a lower prevalence of human infections than other European countries, but it's known that the available data are underestimated, due to underreporting of mild cases and to the absence of an official monitoring programme.

The role of poultry as a reservoir for the transmission of *Campylobacter* to humans has already been recognized, with 20-30% of the human infections linked to handling, preparation, and consumption of broiler meat (EFSA, 2010b). It's known that the risk posed by poultry meat is strongly associated to the presence of high *Campylobacter* loads rather than to its diffusion (EFSA, 2009, 2011; Nauta et al., 2009), and guidelines supplied by EFSA for the harmonized control of Campylobacters in poultry meat underline the importance of a quantitative approach (EFSA, 2008).

With the aim of assessing the exposure to the *Campylobacter* associated risk, the evaluation of poultry meats at retail is critical, as they really enter the consumers' kitchens (Cook et al., 2012). It's known that *Campylobacter* spp. is strongly affected by the environmental conditions, such as oxidative stress, osmotic shock and drying, resulting in a gradual inactivation during production and

storage. So, also if a diffusion of the contamination due to cut and manipulation can happen, lower positivity rates and after all lower counts are often detected at processing level or at retail than earlier in the production chain (Berrang et al., 2001; Uyttendaele et al., 1999). On the other hand, the modern, short processing chain, and the use of protective plastics and dark, moist and cool storage contribute to *Campylobacter* survival, especially in large scale marketing (EFSA, 2009; Harrison et al., 2001). So, high prevalences at retail (60-80%) are sometimes detected, and the presence of some heavily contaminated (>10^4 CFU/g) meat cuts and preparations have been reported by several authors (Humphrey et al., 2007; Suzuki and Yamamoto, 2009; Uyttendaele et al., 2006). Significant differences can be detected among different typologies of poultry meat available on the market, with a decreasing trend from whole carcasses to parts, especially when skin is removed. Due to the inhibitory activity of additives and to the oxidative stress, low frequencies and counts are reported for minced meat and meat preparations, while meat products are considered substantially safe (Cook et al., 2012; Habib et al., 2008; Mena et al., 2008; Meldrum et al., 2006; Uyttendaele et al., 1999, 2006). A particular case is represented by offals (especially livers), that often carry high microbial numbers, leading in some cases to campylobacteriosis outbreaks (Baumgartner et al.,1995; Little et al., 2010; Whyte et al., 2006). Considering the diffusion of *Campylobacter* species, it's known that almost all the isolates coming from poultry meat in Europe belong to *C. jejuni* and *C. coli*, with a general rate of 2/3 and 1/3 of the isolates, respectively, but this ratio varies among countries, and tends to reach a 1/1 value in Southern Europe (EFSA, 2010a; Suzuki and Yamamoto, 2009). Some data suggest the presence of a higher resistance of *C. coli* to environmental conditions, resulting in a relatively higher prevalence of this species in processed meats (Padungtod and Kaneene, 2005).

Official Italian data concerning the prevalence of *Campylobacter* spp. on poultry meats at retail are lacking; the last available studies indicate a variable situation (Nobile et al., 2013; Sammarco et al., 2010). The aim of this study was to collect qualitative and quantitative data concerning the contamination by thermophilic Campylobacters on a wide range of poultry meats marketed in Italy, evaluating the main factors influencing their prevalence and loads.

2. Materials and methods

2.1. Samples selection and experimental design

A total of 472 chicken and turkey meat samples (353 samples of chicken meats, 83 of turkey meats and 36 of mixed meats), including raw sectioned meats, meat preparations and products were

97 submitted to detection and enumeration of *Campylobacter* spp. The samples were obtained from
98 various slaughterhouses, deboning plants and retail sales, located in different Italian regions
99 (Lombardy, Veneto, Tuscany and Sicily) during the period September 2010-June 2013. All the
100 samples were portioned and packed for the retail market. The sampling plan is showed in Table 1.
101 2.2. Sampling and microbiological analyses
102 The samples were withdrawn on the day of preparation for sale/distribution. Each sample was put
103 into sterile stomacher bags (BagLight, Interscience, Saint Nom, F) and transferred to the
104 laboratories, where the analyses were performed within the same day.
105 For the detection of *Campylobacter* spp., the EN ISO 10272-1:2006 method was applied. As
106 requested by the ISO method, the inoculation of mCCDA agar plates (Oxoid, Basingstoke, UK), was
107 combined with another method based on a different principle. The method described by Steele and
108 McDermott (1984), with some modifications, was chosen. An aliquot of 0.5 ml of the enrichment
109 broth was put onto 47 mm diameter, 0.45 mm pore size cellulose membrane filters (Sigma Aldrich
110 Italy, Milan, I) laid on the surface of non-selective blood agar plates (Columbia Agar base added with
111 5% of defibrinated sheep blood, Oxoid). The membranes were left for 45 min and then removed,
112 taking care to avoid the spilling of the broth; the filtered inoculum was spread on the surface by a
113 sterile 10 ml loop. The plates were then incubated at 42 C for 48 h. Typical colonies were isolated
114 by subculturing on Columbia-blood agar and submitted to further confirmation steps.
115 The enumeration of *Campylobacter* spp. in the samples was performed on mCCDA plates by the EN
116 ISO 10272-2:2006 method. For each sample, 5 colonies (when present) were picked, subcultured
117 on blood agar plates incubated 41,5 °C for 24-48 h in microaerobiosis and submitted to
118 confirmation/identification tests.
119 2.3. Identification of the isolates
120 For the identification of the isolates, further steps were performed.
121 Cells morphology and motility were evaluated by microscope observation (1000X magnification) of
122 a suspension of the isolates in 1 ml of Brucella broth (Oxoid); oxidase determination (Oxidase strips,
123 Oxoid) and Gram staining were also performed. The isolates were then subcultured in two series of
124 blood agar plates, one incubated at 41.5 °C for 44 ± 4 h in aerobiosis, and the other incubated at 25
125 °C for 44 ± 4 h in microaerobic atmosphere. For the provisional identification of the species, the
126 isolates showing the typical characteristics of thermophilic Campylobacter spp. (little curve Gram
127 negative rods with corkscrew motility, unable to grow in aerobiosis or at 25 C, oxidase positive) were
128 evaluated for the catalase activity and the susceptibility to cephalotin (30 µg) and nalidixic acid (30

μg) (Oxoid) by the disk diffusion method, and were submitted to the identification by API Campy kit (bioMerieuxItalia, Bagno a Ripoli, I).

In the further identification step, the isolates were prepared, properly labelled and sent from each research unit to a unique laboratory for the biomolecular identification. The strains were inoculated onto blood agar plates and incubated at 41,5 ± 1 àC for 48 h in microaerobic conditions; bacterial slime was recovered by a sterile swab, that was inserted into a tube of Amies transport medium with charcoal (Oxoid). For DNA extraction, the strains were also inoculated in Brain Heart Infusion broth (BHI, Oxoid) added with 5% of laked horse blood and 15% of glycerol, and stored at -80 C until the analysis. For the identification of the genera (*Campylobacte*r, *Helycobacter*, *Arcobacter*), a simplex PCR-RFLP analysis of the 16S rRNA Gene was performed, following the method described by Marshall et al. (1999). The isolates identified as *Campylobacter* spp. where then submitted to species identification by a multiplex PCR method, as described by Wang et al. (2002).

2.4. Statistical analysis

All the data obtained from qualitative analyses were submitted to the frequency distribution analysis (chi square test) considering the following factors: sample typology, supplier typology, meat species and sampling season. The differences among the counts obtained considering the same factors were also evaluated, using the SAS/stat package version 8.0 (SAS Inst. Inc., Cary, NC). A value of $P < 0.05$ was considered statistically significant.

3. Results and discussion

3.1. Prevalence of *Campylobacter* spp. in poultry meats

A total of 472 samples of chicken and turkey meat and products thereof were analyzed for the presence of presumptive *Campylobacter* spp. The presence of these microorganisms was revealed in 161 samples (34.1%). A comparison with previous Italian data was made: the most extensive data set concerning the prevalence and count of *Campylobacter* spp. on poultry meat in Italy comes from the EU base line survey (EFSA, 2010a), that evidenced a prevalence of 49.6% contaminated broiler carcasses, with 12.5% hosting counts higher than 3 Log CFU/g. Considering the similar samples analyzed in our study, that is sectioned meats with skin taken at the slaughterhouse, a higher prevalence was obtained (more than 85%), but without high counts (see Table 2). These data can be explained by the differences between the two sampling plans: at first, in the EU base line survey, only whole carcasses were sampled, while in our study also cuts (wings, thighs, etc.) were analyzed, with a likely contamination during cutting (by the equipments or workers' hands). Moreover, in this

study, the samples were taken just before the expedition to the retailers (in the EU survey samples were taken just after chilling), with a longer exposition to low environmental temperatures, so resulting in decreased counts.

The results coming from previous Italian studies performed on different poultry meats at retail, that are easily comparable with our data, indicate a high prevalence of thermophilic campylobacters, with very variable values (20-80%) (Nobile et al., 2013; Pezzotti et al., 2003; Parisi et al., 2007; Sammarco et al., 2010; Zanetti et al., 1996). A possible decreasing trend was hypothesized by Nobile et al. (2013), due to an increasing care for contamination of carcasses with the aim of reducing *Salmonella* prevalence, but no official surveillance information is available in Italy. As stated by the EFSA reports, to characterize the real risk for consumers, it is critical to consider the quantitative data rather than the raw prevalence of Campylobacters (Nauta et al., 2009), as it is known that a reduction of counts on poultry carcass surfaces could result in a significant reduction of human campylobacteriosis cases (50% and 90% reduction with a threshold value of 1000 or 500 CFU/ g of skin, respectively) (EFSA, 2011). In this study, almost the 70% of samples with detectable counts showed values lower than 1 Log CFU/g, that was the technical limit of the method; the percentage rose to 86.8% and 97.1% considering a level lower than 2 and 3 Log CFU/g, respectively. These data give a picture of a high probability of potential presence of *Campylobacter* in portioned meats that can enter the kitchen, also if with a low risk of high counts due to undercooking/cross contamination.

3.2. Influence of meat typology on the contamination rate

Due to the behaviour of Campylobacters, it is particularly important to consider the strong influence of different production/ preparation methods on the survival of these microorganisms, leading to different scenarios among the wide range of different poultry meats present on the market (whole, minced, cooked, etc.).

The highest prevalence was detected in sectioned meats and offals, with about half of the samples carrying *Campylobacter* spp. (Fig. 1). The rates observed in these two typologies of samples were significantly higher ($P < 0.01$) than those observed in meat preparations (about 25% of prevalence) and in meat products (only 4%). Quantitative data gave a similar trend, as the rate of samples with a very low level of contamination (<1 Log CFU/g) was 58.5% and 88.5% for sectioned meats and meat preparations, respectively (Fig. 2). These results confirm the decreasing trend of *Campylobacter* prevalence along the production process, as these microorganisms are particularly sensitive to environmental inactivating agents, such as oxidative stress and drying of meat. The

frequent contamination of offals was in agreement with the data obtained in other European countries (Mackiw et al., 2011; Mena et al., 2008). No differences were observed in *Campylobacter* spp. prevalence among combs (50%), gizzards (47,4%) and livers/hearts (50%), also if the number of samples was too low to perform a statistical analysis. The quantitative data gave a variable picture: a general low level of contamination was observed (almost 70% of the samples < 2 Log CFU/g, and 52.6% < 1 Log CFU/g), but the positive liver samples were evidently more contaminated than the other typologies, with 4/4 counts > 3 Log CFU/g and a maximum value of 4.04 Log CFU/g. These results agree with those of previous studies, showing an extremely high contamination rate of the livers and the presence of samples with counts higher than 4 Log (Baumgartner et al., 1995; Whyte et al., 2006). Also if its diffusion among consumers is lower than other frequently contaminated poultry meats (e.g. sectioned cuts with skin), the liver is known as a potentially high risk source for the consumer, due to the potential cross contamination in the kitchen or to the infrequent but hazardous preparation of raw products like pates (Little et al., 2010).

Considering the sectioned meats, a significantly higher prevalence ($P < 0.01$) was observed in skin-on meats (86.8%, 46/53 positive samples) than in skin-off samples, represented mainly by breasts (32.7%, 34/104 positive samples). These data were expected, due to the contamination route of *Campylobacter* spp. on poultry carcasses (mainly during scalding and defeathering operations), and were confirmed by the quantitative results, showing a double rate of samples below 1 Log CFU/g in skin-off meats (84.6 vs 41%). The decrease of the prevalence of *Campylobacter* spp. in meat samples without skin has been constantly reported, also if this difference is very variable (Baumgartner and Felleisen, 2011; Cook et al., 2012; Uyttendaele et al., 1999). Also if the contamination of the cuts without skin likely occurs during the sectioning activity, a smaller contamination extent, associated to a lower survival ability on meat than on skin, often results in a complete inactivation of Campylobacters on the surface (Berrang et al., 2001; Davis and Conner, 2007); thus, the absence of skin can be considered an important factor for risk reduction. Among skin-on sectioned meats no significant differences were observed in the different cuts such as wings, legs, thighs, drumsticks and half carcasses. A higher contamination was observed in legs, thighs and wings (about 30% of counts > 2 Log CFU/g) if compared to drumsticks (no samples > 2 Log); this slight difference could be due to the position of the carcass at the slaughterhouse (head back hanged), but different results were obtained by other authors (Habib et al., 2008). Among skin-off samples, a significantly higher prevalence ($P < 0.05$) was observed in breast samples, both whole or sliced (45.2 and 46.4%, respectively) towards the other typologies (stew and leg meat, with 10.5% and 19.2%, respectively).

Due to the lower preparation time and exposure to air, the whole breast samples showed higher counts (all above 1 Log CFU/g) than sliced or pieced meats. The contamination of breasts is particularly important for consumers, as this typology of meat is one of the most consumed and the cross contamination in the kitchen likely occurs during preparation/slicing; for this reason, several studies have been conducted showing a wide range of positivity rates (30-87%) and counts (until 4 Log CFU/g) (Habib et al., 2008; Hamedy et al., 2007; Luber and Bertelt, 2007). Considering meat preparations, a significantly lower prevalence ($P < 0.01$) was revealed in samples produced with minced meat (22.4%) than in those obtained from whole pieces (58.3%). As already supposed by Suzuki and Yamamoto (2009) during grinding the wider exposition to the oxidative stress could affect Campylobacters survival. As expected, a higher rate of very low counts (<1 Log CFU/g) was detected in minced meat samples (92.7 vs 72.7%). This difference has been observed also in other European studies, suggesting also the importance of the previous exposition to air of meat cuts prior to grinding (Baumgartner and Felleisen, 2011; Habib et al., 2008; Mackiw et al., 2011; Mena et al., 2008). No significant differences were detected among specific minced meat preparations, but a higher rate was detected in hamburgers and sausages (about 20%) than in meat patties (12.5%), produced with a larger amount of other ingredients.

Meat products showed a very low positivity rate; with only two positive rolled raw meat samples, while all the thermally processed products (frankfurters) gave negative results. It is generally known that meat products don't represent a real risk source for consumers, due to the thermal sensitivity of *Campylobacter* spp. on the meat surface, with a D-value of 1 min at 60 C (Jacobs-Reitsma, 2000; Mackiw et al., 2011).

3.3. Influence of the plant typology

Considering the general prevalence of thermophilic Campylobacters in the samples taken from plants working in different production stages, the data evidenced a clearly higher positivity rate in meats withdrawn at slaughterhouse (about 80%), presumably due to the lower time of exposure to the environmental stresses; the rate decreased in samples from DP (49%) and retailers (37 and 25% for BS and LSR, respectively). As the main factor influencing the contamination is the product typology, the raw influence of the plant typology was statistically evaluated on samples that were purchased in at least two different typologies. The data concerning the samples taken from the different plant categories are shown in Table 2. Liver samples purchased at SL were evidently more contaminated than those taken from LSR, considering both the positivity rate than the counts: all the samples taken from slaughterhouse but one had counts above 3 Log CFU/g, while all the counts

of samples from LRS were lower than 1 Log CFU/g. For skin-on sectioned meats, the positivity rate was very high for all the typologies of suppliers, but important differences in the counts were obtained, with only 3/23 samples above 1 Log at slaughterhouse, 7/8 in deboning plants and 6/8 in large scale retails. The difference among the suppliers was more marked in skin-off sectioned meats, as a strong decrease in the positivity rate was observed following the production chain; counts higher than 1 Log were detected only in samples from the slaughterhouses. It was thus evidenced the importance of marketing/exposition time on the contamination during the first post-slaughtering phases; in order to investigate this aspect, the data obtained from sectioned meats samples taken at the slaughterhouses were divided considering the interval (0, 1, 2, 3 days) between slaughtering and distribution. The positivity rate was extremely high during the first two days (mean value of 96%), while it decreased significantly ($P < 0.01$) in samples packaged 3 or more days after slaughtering (40%). No significant differences were revealed among the counts, with similar median values (1e2 Log CFU/g), but a higher rate of values above 2 Log CFU/g in meats distributed 0-1 days after slaughtering was detected (36.7% vs 8.3%).

Considering whole meat preparations, a higher positivity rate was observed for BS than for LSR, without differences in quantitative data; in minced meat preparations, very similar results were observed both for positivity rate and counts between BS and LSR. Data from other studies gave controversial results, in particular regarding the differences between traditional shops and large scale retails, due to different marketing conditions (exposure time, manipulation practices, ecc.) (Harrison et al., 2001; Meldrum et al., 2006).

3.4. Influence of meat species and 3.4. Influence of meat species and sampling season

A comparison between chicken and turkey meats was made for minced meat preparations and skin-on sectioned meats; the positivity rate in chicken samples was significantly higher ($P < 0.05$), for both sectioned meats (40.0 vs. 21.1%) and minced meat preparations (23.4 vs. 14.0%). Similar results were obtained by other authors, in particular for skin-on meats (Korsak et al., 2015; Moran et al., 2009; Uyttendaele et al., 1999; Whyte et al., 2004). As the prevalence of *Campylobacter* spp. in broiler population is known to be lower than in turkeys (EFSA-ECDC, 2015), the higher positivity rate in chicken meats can be due to the slaughtering process, linked to a higher animal density (e.g. during transport, scalding or defeathering, evisceration) and to the different methods applied, for example during stunning (as the water bath for electronarcosis is still often used only for broilers), and scalding. In particular, “hard scalding” at temperatures around 60 °C is mainly applied on heavy animals such as turkeys (Zhuang et al., 2013), causing an important decrease in *Campylobacter*

numbers in the scalding water (Oosterom et al., 1983), thus resulting in a lower contamination of the carcasses. Some authors hypothesized also a lower tendency of turkey skin to harbour microorganisms than the chicken one, due to its smoother surface (Acuff et al., 1986).

Considering the trend of positivity rate among the sampling seasons, significantly higher rates in summer/spring were evidenced in minced meat preparations (Table 3), also if the counts were substantially similar. No differences were revealed for skin-on sectioned meats, as extremely high rates were constant; in skin-off samples, slight differences were revealed with higher rates in summer, but without a clear trend. A seasonality in the prevalence of *Campylobacter* spp. in broiler carcasses was observed in Italy by Manfreda et al. (2006), but the climatic influence is evidently more marked in Northern European countries (Boysen et al., 2011; EFSA, 2010a). The peak in the warm season, evidenced in broiler meats, is known to occur parallel to the peak of human campylobacteriosis cases, also if the link has not been completely cleared and the influence of common environmental factors was suggested (Jore et al., 2010; Meldrum et al., 2005).

3.5. Diffusion of *Campylobacter* species

A total of 367 provisional *Campylobacter* spp. isolates were submitted to multiplex PCR; for 357 isolates, a successful species identification was obtained. Almost all the strains (about 99%) belonged to the species *C. jejuni*/*C. coli*, while only 3 isolates were identified as *C. lari* and one as *C. mucosalis*. These data agree with previous observations, showing the very high relative frequency of the two main species in industrialized countries (Williams and Oyarzabal, 2012). The relative prevalences among the identified isolates were very similar, with a small predominance of *C. coli* (186 isolates, 52.1%) if compared to *C. jejuni* (167 isolates, 46.8%). Previous results obtained from Italian retail meat samples showed a higher frequency of *C. jejuni* (Pezzotti et al., 2003; Sammarco et al., 2010; Nobile et al., 2013), but, as in our case, the difference was not very evident. Data from other European countries show the effect of latitude on the relative frequency of the two species, with a marked preponderance of *C. jejuni* in Northern Europe (up to 100% on carcasses in the Baltic countries and 70e85% of positive retail samples in northwestern Europe), and a gradual increase of *C. coli* prevalence going South, reaching an equal distribution in Mediterranean countries (EFSA, 2010a; Habib et al., 2008; Moran et al., 2009; Whyte et al., 2004).

The contamination rates of *C. jejuni* and *C. coli* in the samples that gave positive results are reported in Table 4. Considering the whole set of data, a higher prevalence was observed for *C. coli* (58.4%) than for *C. jejuni* (57.1%), without statistically significant differences.

The picture obtained from the different typologies of meat samples was variable: *C. coli* was significantly more frequently isolated than *C. jejuni* in offal samples (mainly livers), while in the other typologies (sectioned meats and meat preparations) the isolation rates of the two species were very similar. Considering only the sectioned meat samples taken at the slaughterhouse, no influence of the duration of the slaughtering-sampling interval was detected. For meat products, no comparison were made due to the very little number of identified isolates (only one *C. jejuni* and one *C. coli*). A greater ability of *C. coli* to survive in unfavourable environmental conditions has been described by some authors; this characteristic can result in a predominance of this species in productive plants and in an increasing relative frequency during the preparation of meats (Damjanova et al., 2011; Padungtod and Kaneene, 2005). Our data agree with these observations, as the positivity rate of *C. jejuni* was significantly higher in samples from SL and DP, while the opposite situation was evidenced in samples from BS and LSR. The higher relative prevalence of *C. coli* in samples taken during winter and spring was unexpected, as the few Italian available data suggested a relatively better resistance of *C. jejuni* to low environmental temperatures (Manfreda et al., 2006).

Finally, considering the relative diffusion in chicken and turkey meat samples, our data showed the higher rate of *C. coli* in chicken samples, confirming the results of a previous study by Nobile et al. (2013) and the findings of the EU base line study (EFSA, 2010a). The predominance of *C. jejuni* in turkey meat samples, described by Hamedy et al. (2007) and Nobile et al. (2013) was not observed in this case. It has to be noted that, as suggested by some authors, it is difficult to isolate the influence of single factors, as the relative frequencies of *Campylobacter* species are affected by multiple factors such as plant, product, state and storage of meats.

4. Conclusions

The surveillance of the prevalence of *Campylobacter* spp. In poultry meat is nowadays insufficient due to the absence of routine control programs in several countries, including Italy. Possible future programs should take into account the suggestions reported in EFSA guidelines, stressing the need for a link between control measures and real risk for consumers. In particular, retail poultry meats seems to be a proper target, as they are directly acquired, manipulated and prepared by the consumers. As observed in our study, the risk categorization should mainly consider the typology of meats (raw meats with/without skin, meat preparations), avoiding the thermally processed meat products, that are substantially free of *Campylobacter* spp. An efficient control program must supply quantitative data, as it is known that a little number of highly contaminated meat samples are

responsible of almost all the human campylobacteriosis cases. The picture supplied by our data indicate the extremely low frequency of high counts, that are linked to specific meat typologies, such as liver. To obtain a complete map of the risks, in addition to the production/ processing factors included in this study, other elements should be taken into account, such as consumers behaviour and consumption patterns, in particular in a varied population. So, interventions in consumers' education, to enhance the importance of correct preparation practices should be considered as an essential tool by the authorities.

References

Acuff, G.R., Vanderzant, C., Gardner, F.A., Golan, F.A., 1986. Prevalence of *Campylobacter jejuni* in turkey carcass processing and further processing of turkey products. J. Food Prot. 49, 712e717.

Baumgartner, A., Felleisen, R., 2011. Market surveillance for contamination with thermotolerant Campylobacters on various categories of chicken meat in Switzerland. J. Food Prot. 74 (12), 2048e2054.

Baumgartner, A., Grand, M., Liniger, M., Simmen, A., 1995. *Campylobacter* contaminations of poultry liver-consequences for food handler and consumers. Arch. Leb. 46, 11e12.

Berrang, M.E., Ladely, S.R., Buhr, R.J., 2001. Presence and level of *Campylobacter*, coliforms, *Escherichia coli*, and total aerobic bacteria recovered from broiler parts with and without skin. J. Food Prot. 64 (2), 184e188.

Boysen, L., Vigre, H., Rosenquist, H., 2011. Seasonal influence on the prevalence of thermotolerant *Campylobacter* in retail broiler meat in Denmark. Food Microbiol. 28, 1028e1032.

Cook, A., Odumeru, J., Lee, S., Pollari, F., 2012. *Campylobacter*, *Salmonella*, *Listeria monocytogenes*, verotoxigenic *Escherichia coli*, and *Escherichia coli* prevalence, enumeration, and subtypes on retail chicken breasts with and without skin. J. Food Prot. 75 (1), 34e40.

Damjanova, I., Jakab, M., Farkas, T., Meszaros, J., Galantai, Zs, Turcsanyi, I., Bistyak, A., Juhasz, A., Paszti, J., Kiss, I., Kardos, G., 2011. Form farm to fork follow-up of thermotolerant campylobacters throughout the broiler production chain and in human cases in a Hungarian county during a ten-months period. Int. J. Food Microbiol. 150, 95e102.

Davis, M.A., Conner, D.E., 2007. Survival of *Campylobacter jejuni* on poultry skin and meat at varying temperatures. Poult. Sci. 86 (4), 765e767.

EFSA (European Food Safety Authority), 2008. Report of Task Force on Zoonoses Data Collection on proposed technical specifications for a coordinated monitoring programme for *Salmonell*a and *Campylobacter* in broiler meats at retail in the EU. EFSA J. 155, 1.

EFSA (European Food Safety Authority), 2009. Assessing Health Benefits of Controlling *Campylobacte*r in the Food Chain. Summary report EFSA Scientific Colloquium 12, 4e5/12 2008, Rome, Italy. Available at: http://www.efsa.europa.eu/it/events/event/colloque081204-m.pdf (Accessed 27 April 2015).

EFSA (European Food Safety Authority), 2010a. Analysis of the baseline survey on the prevalence of *Campylobacter* in broiler batches and of *Campylobacter* and *Salmonella* on broiler carcasses in the EU, 2008, Part A: *Campylobacter* and *Salmonella* prevalence estimates. EFSA J. 8 (3), 1503.

EFSA (European Food Safety Authority), 2010b. Panel on Biological Hazards (BIOHAZ), Scientific Opinion on quantification of the risk posed by broiler meat to human campylobacteriosis in the EU. EFSA J. 8 (1), 1437.

EFSA (European Food Safety Authority), 2011. Panel on Biological Hazards (BIOHAZ); Scientific Opinion on *Campylobacter* in broiler meat production: control options and performance objectives and/or targets at different stages of the food chain. EFSA J. 9 (4), 2105.

EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control), 2015. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2014. EFSA J. 13 (12), 4329.

Habib, I., Sampers, I., Uyttendaele, M., Berkvens, D., De Zutter, L., 2008. Baseline data from a Belgium-wide survey of *Campylobacter* species contamination in chicken meat preparations and considerations for a reliable monitoring program. Appl. Environ. Microb. 74, 5483e5489.

Hamedy, A., Ludewig, M., Fehlhaber, K., Alter, T., Schlichting, D., 2007. Quantitative detection of *Campylobacter* spp. on turkey carcasses and turkey meat. Fleischwirtschaft 87 (10), 121e124.

Harrison, W.A., Griffith, C.J., Tennant, D., Peters, A.C., 2001. Incidence of *Campylobacter* and *Salmonella* isolated from retail chicken and associated packaging in South Wales. Lett. Appl. Microbiol. 33, 450e454.

Humphrey, T., O'Brien, S., Madsen, M., 2007. Campylobacters as zoonotic pathogens: a food production perspective. Int. J. Food Microbiol. 117, 237e257.

ISO (International Organization for Standardisation), 2006a. Microbiology of Food and Animal Feeding Stuffs e Horizontal Method for Detection and Enumeration of *Campylobacter* spp. Part 1: Detection Method. ISO 10272-1:2006. ISO, Geneva, Switzerland.

ISO (International Organization for Standardisation), 2006b. Microbiology of Food and Animal Feeding Stuffs e Horizontal Method for Detection and Enumeration of *Campylobacter* spp. Part 2: Colony-count Technique. ISO 10272-2:2006. ISO, Geneva, Switzerland.

Jacobs-Reitsma, W., 2000. *Campylobacter* in the food supply. In: Nachamkin, I., Blaser, M.J. (Eds.), Campylobacter, second ed. ASM Press, Washington, DC, pp. 467e481.

Jore, S., Viljugrein, H., Brun, E., Heier, B.T., Borck, B., Ethelberg, S., Hakkinen, M., Kuusi, M., Reiersen, J., Hansson, I., Olsson Engvall, E., Løfdahl, M., Wagenaar, J.A., van Pelt, W., Hofshagen, M., 2010. Trends in *Campylobacter* incidence in broilers and humans in six European countries, 1997-2007. Prev. Vet. Med. 93, 33e41.

Korsak, D., Mackiw, E., Roz_ynek, E., Zyłowska, M., 2015. Prevalence of *Campylobacter* spp. in retail chicken, turkey, pork and beef meat in Poland between 2009 and 2013. J. Food Prot. 78, 1024e1028.

Little, C.L., Gormley, F.J., Rawal, N., Richardson, J.F., 2010. A recipe for disaster: outbreaks of campylobacteriosis associated with poultry liver pate in England and Wales. Epidemiol. Infect. 138, 1691e1694.

Luber, P., Bertelt, E., 2007. Enumeration of *Campylobacter* spp. on the surface and within chicken breast fillets. J. Appl. Microbiol. 102, 313e318.

Mackiw, E., Szewuska, K., Stos, K., Jarosz, M., Korsak, D., 2011. Occurrence of *Campylobacter* spp. in poultry and poultry products for sale on the Polish retail market. J. Food Prot. 74 (6), 986e989.

Manfreda, G., De Cesare, A., Bondioli, V., Stern, N.J., Franchini, A., 2006. Enumeration and identity of *Campylobacter* spp. in Italian broilers. Poult. Sci. 85, 556e562.

Marshall, S., Melito, P., Woodward, D., Johnson, W., Rodgers, F., Mulvey, M., 1999. Rapid identification of *Campylobacter*, *Arcobacter* and *Helicobacter* isolates by PCR-restriction fragment length polymorphism analysis of the 16S rRNA gene. J. Clin. Microbiol. 37 (12), 4156e4160.

Meldrum, R.J., Griffiths, J.K., Smith, R.M.M., Evans, M.R., 2005. The seasonality of human campylobacter infection and *Campylobacter* isolates from fresh, retail chicken in Wales. Epidemiol. Infect. 133, 49e52.

Meldrum, R.J., Smith, R.M.M., Wilson, I.G., 2006. Three-year surveillance program examining the prevalence of *Campylobacter* and *Salmonella* in whole retail raw chicken. J. Food Prot. 69 (4), 928e931.

Mena, C., Rodrigues, D., Silva, J., Gibbs, P., Teixeira, P., 2008. Occurrence, identification and characterization of *Campylobacter* species isolated from Portoguese poultry samples collected from retail establishments. Poult. Sci. 87, 187e190.

446 Moran, L., Scates, P., Madden, R., 2009. Prevalence of *Campylobacter* spp. in raw retail poultry on
447 sale in Northern Ireland. J. Food Prot. 72 (9), 1830e1835.
448 Nauta, M., Hill, A., Rosenquist, H., Brynestad, S., Fetsch, A., van der Logt, P., Fazil, A., Christensen,
449 B., Katsma, E., Borck, B., Havelaar, A., 2009. A comparison of risk assessments on *Campylobacter* in
450 broiler meat. Int. J. Food Microbiol. 129, 107e123.
451 Nobile, C.G.A., Costantino, R., Bianco, A., Pileggi, C., Pavia, M., 2013. Prevalence and pattern of
452 antibiotic resistance of *Campylobacter* spp. in poultry meat in Southern Italy. Food Control 32,
453 715e718.
454 Oosterom, J., Notermans, S., Karman, H., Engels, G.B., 1983. Origin and prevalence of
455 Campylobacter jejuni in poultry processing. J. Food Prot. 46, 339e344.
456 Padungtod, P., Kaneene, J.B., 2005. *Campylobacter* in food animals and humans in northern
457 Thailand. J. Food Prot. 68 (12), 2519e2526.
458 Parisi, A., Lanzilotta, S.G., Addante, N., Normanno, G., Di Modugno, G., Dambrosio, A., Montagna,
459 C.O., 2007. Prevalence, molecular characterization and antimicrobial resistance of thermophilic
460 *Campylobacter* isolates from cattle, hens broilers and broiler meat in south-eastern Italy. Vet. Res.
461 Commun. 31, 113e123.
462 Pezzotti, G., Serafin, A., Luzzi, I., Mioni, R., Milan, M., Perin, R., 2003. Occurrence and resistance to
463 antibiotics of *Campylobacter jejuni* and *Campylobacter coli* in animals and meat in northeastern
464 Italy. Int. J. Food Microbiol. 82, 281e287.
465 Sammarco, M.L., Ripabelli, G., Fanelli, I., Grasso, G.M., Tamburro, M., 2010. Prevalence and
466 biomolecular characterization of *Campylobacter* spp. isolated from retail meat. J. Food Prot. 73 (4),
467 720e728.
468 Steele, T.W., McDermott, S.N., 1984. The use of membrane filters applied directly to the surface of
469 agar plates for the isolation of *Campylobacter jejuni* from feces. Pathology 16 (3), 263e265.
470 Suzuki, H., Yamamoto, S., 2009. *Campylobacter* contamination in retail poultry meats and by-
471 products in the world: a literature survey. J. Vet. Med. Sci. 71, 255e261.
472 Uyttendaele, M., Baert, K., Ghafir, Y., Daube, G., De Zutter, L., Herman, L., Dierick, K., Pierard, D.,
473 Dubois, J.J., Horion, B., Debevere, J., 2006. Quantitative risk assessment of *Campylobacter* spp. in
474 poultry based meat preparations as one of the factors to support the development of risk-based
475 microbiological criteria in Belgium. Int. J. Food Microbiol. 111, 149e163.

476 Uyttendaele, M., De Troy, P., Debevere, J., 1999. Incidence of *Salmonella*, *Campylobacter jejuni*,
477 *Campylobacter coli*, and *Listeria monocytogenes* in poultry carcasses and different types of poultry
478 products for sale on the Belgian retail market. J. Food Prot. 62 (7), 735e740.
479 Wang, G., Clark, C.G., Taylor, T.M., Pucknell, C., Barton, C., Price, L., Odward, D.L., Rogers, F.G., 2002.
480 Colony multiplex PCR assay for identification and differentiation of *Campylobacter jejuni*, *C. coli*, *C.*
481 *lari, C. upsaliensis*, *C.fetus* subsp. *fetus*. J. Clin. Microbiol. 40, 4744e4747.
482 Whyte, R., Hudson, J.A., Graham, C., 2006. *Campylobacter* in chicken livers and their destruction by
483 pan frying. Lett. Appl. Microbiol. 43, 591e595.
484 Whyte, P., McGill, K., Cowley, D., Madden, R.H., Moran, L., Scates, P., Carroll, C., O'Leary, A., Fanning,
485 S., Collins, J.D., McNamara, E., Moore, J.E., Cormican, M., 2004. Occurrence of *Campylobacter* in
486 retail foods in Ireland. Int. J. Food Microbiol. 95, 111e118.
487 Williams, A., Oyarzabal, O.A., 2012. Prevalence of *Campylobacter* spp. in skinless, boneless retail
488 broiler meat from 2005 through 2011 in Alabama, USA. BMC Microbiol. 12, 184.
489 Zanetti, F., Varoli, O., Stampi, S., De Luca, G., 1996. Prevalence of thermophilic *Campylobacter* and
490 *Arcobacter butzleri* in food of animal origin. Int. J. Food Microbiol. 33 (2e3), 315e321.
491 Zhuang, H., Bowker, B.C., Buhr, R.J., Bourassa, D.V., Kiepper, B.H., 2013. Effects of broiler carcass
492 scalding and chilling methods on quality of early-deboned breast fillets. Poult. Sci. 92, 1393e1399.
493
494

Table 1

Table 1
Sampling plan for the evaluation of the prevalence and quantification of *Campylobacter* spp. in poultry meat and products thereof. SL: slaughterhouse; DP: deboning plant; BS: butcher's shop; LSR: large scale retailer.

Meat typology	Plant typology (no of samples)
Skin-on sectioned meats (half carcasses, legs, thighs, drumsticks, wings)	SL (27), DP (8), LSR (18)
Skin-off sectioned meats (whole breast, sliced breasts, leg meat, stew)	SL (9), DP (33), LSR (62)
Offals (livers/hearts, gizzards, combs)	SL (16), LSR (23)
Whole-pieces meat preparations (rolls, breaded breast slices, spitted meat)	BS (10), LSR (14)
Minced meat preparations (hamburgers, raw sausages, patties)	BS (31), LSR (174)
Meat products (wurstels, rolls)	LSR (47)

501

502

503 Table 2

504

Table 2
Comparison of the data from different plant typologies. SL: slaughterhouse, DB: deboning plant, BS: butcher's shop, LSR: large scale retail. Median values are expressed as Log CFU/g.

Meat typology	SL		DP		BS		LSR	
	Positivity rate (%)	Median value	Positivity rate (%)	Median value	Positivity rate (%)	Median value	Positivity rate (%)	Median value
Liver	71.4	3.60	–	–	–	–	33.3	<1
Skin-on sectioned meats	85.2	1.74	100	<1	–	–	83.3	<1
Skin-off sectioned meats	88.9	1.48	36.4	<1	–	–	22.6	<1
Whole meat preparations	–	–	–	–	70.0	<1	50.0	<1
Minced meat preparations	–	–	–	–	25.8	<1	21.8	<1

505

506

507

508

509

510 Table 3

511

Table 3
Comparison of positivity rates during the sampling seasons. Values in the same row with different letters are significantly different (^{a,b} $P < 0.05$; ^{A,B} $P < 0.01$).

Meat typology	Spring	Summer	Autumn	Winter
Skin-on sectioned meats (n° positive/total samples)	71.4% (5/7)	100% (15/15)	86.7% (13/15)	81.25% (13/16)
Skin-off sectioned meats (n° positive/total samples)	22.2% (4/18)	39.1% (9/23)	30.8% (8/26)	35.1% (13/37)
Minced meat preparations (n° positive/total samples)	21.1%^{a} (4/19)	38.0%^{A} (38/100)	6.0%^{B} (3/50)	2.8%^{B,b} (1/36)

512

513

514

515

516

517 Table 4

518

Table 4
Comparison of positivity rates of *C. jejuni* and *C. coli* in the *Campylobacter* spp. positive samples. Values in the same row with different letters are significantly different ($^{a,b}P < 0.05$; $^{A,B}P < 0.01$).

		C. jejuni	*C. coli*
Sample typology	Offals	26.3%^{b}	68.4%^{a}
	Sectioned meats	60.0%	65.0%
	Meat preparations	55.0%	56.7%
Plant typology	SL - DP	72.6%^{a}	50.0%^{b}
	BS - LSR	47.5%^{b}	63.6%^{a}
Sampling season	Spring	26.7%^{B}	86.7%^{A}
	Summer	62.5%	53.8%
	Autumn	57.1%	64.3%
	Winter	13.2%^{B}	86.8%^{A}
Species	Chicken	51.9%	63.9%
	Turkey	56.3%	56.3%

519

520

521

522

523

524 Figure 1

525

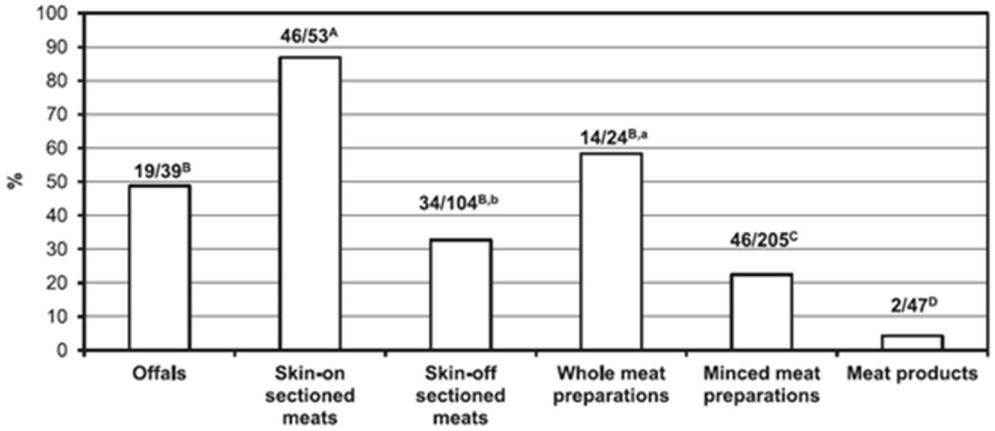

Fig. 1. Prevalence of *Campylobacter* spp. in the different meat sample typologies.

526

527

528

529

530

531 Figure 2

532

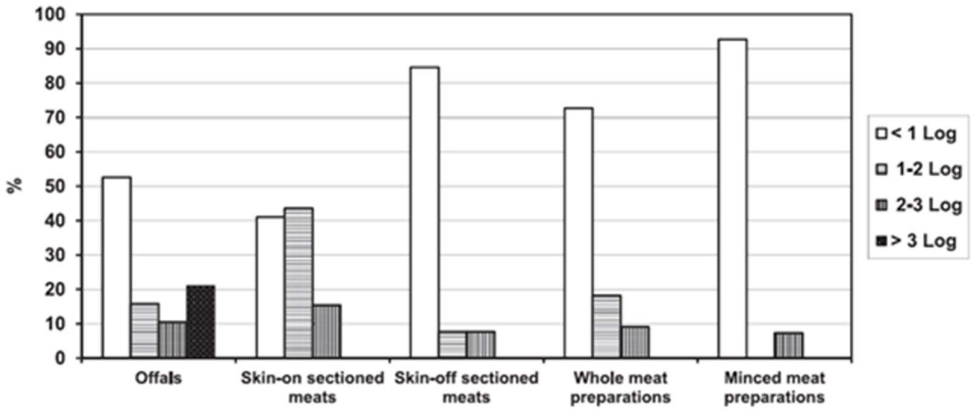

Fig. 2. Frequency distribution of *Campylobacter* spp. counts in the different meat typologies.

533

534