Occurrence of thermotolerant Campylobacter in raw poultry meat, environmental and pigeon stools collected in open-air markets

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Introduction

Thermophilic Campylobacter, especially Campylobacter jejuni and Campylobacter coli, are considered the most common bacterial agent of human gastroenteritis in developed countries. Directive 2003/99/EC (European Commission, 2003) establishes the monitoring of zoonoses and zoonotic agent such as Campylobacter spp. This approach shall include each production step in the food chain: from farm level to the retail sale. Campylobacter jejuni and Campylobacter coli are distributed in the intestinal tract of many asymptomatic birds and mammals that are considered the main reservoirs for these microorganisms. Transmission to humans mainly occurs through consumption or handling of animal origin foods; moreover, contact with infected animals may determine human campylobacteriosis (OIE, 2008). The disease is usually characterised by acute gastrointestinal signs represented by diarrhea, abdominal pain, nausea, vomiting, fever and headache. Symptoms occur one to five days after infection and last for several days. Campylobacteriosis cases may be rarely associated to reactive arthritis, Guillain-Barré syndrome, nephritis and hepatitis. In the European Union (EU), human campylobacteriosis is increasing with a statistically significant rate of confirmed cases from 2008 to 2011 among 13 reporting Member States. In 2011 confirmed cases of human campylobacteriosis in EU were 200,209 (EPSA, 2013): this positive trend was characterised by a clear seasonal factor. Human cases were reported more frequently during summer months, when ambient temperature is higher. In fact, a study conducted in the EU showed that the season can represent an important risk factor for poultry contamination [OR=7.4, CI (9.3:536) in the period from July to September compared with January-March] (EFSA, 2011). In addition to summer temperatures, it must be considered that the high number of flies (from July to September) could represent a mechanical vector for contamination of poultry meat (Hald et al., 2008).

Actually, raw chicken meat is considered the major source of human campylobacteriosis in Europe. Surveys conducted at retail level, often showed high contamination values by thermophilic Campylobacter: 38.4% (435/1132) in fresh meat and meat products of poultry in Switzerland (Baumgartner and Felleisen, 2011), 87.2% (321/368) in raw poultry meat, 84 chicken and 2 turkey meat samples collected at some supermarkets in Lithuania (Kudirkienė et al., 2013).

Campylobacteriosis was the most commonly reported zoonosis for confirmed human cases in European Union during 2011. Poultry meat was very often implicated in Campylobacter infections in humans. In Italy commerce of raw poultry meat is common in open-air markets: these areas can be considered at high risk of bacterial contamination due to the high presence birds like pigeons. The aim of this study was to collect data about the contamination by thermotolerant Campylobacter of raw poultry meat commercialised in open-air markets, of work-surfaces in contact with poultry meat and of pigeon stools sampled in the market areas in Turin, Northern Italy. Between September 2011 and December 2012, 86 raw poultry meat samples, 86 environmental swabs and 108 animal samples were collected in 38 open-air markets. Analysis were carried out according to ISO10272-1:2006 standard. C. coli was detected in 2.3% (2/86) of raw poultry meat samples, whereas no swab (0/86) resulted positive. Of pigeon stool 28% (30/107) was positive for C jejuni (83.3% C jejuni subsp. jejuni and 16.7% C jejuni subsp. doylei). C jejuni subsp. jejuni was isolated from 1 dead pigeon. Our results showed lower rates of contamination than those reported at retail in Europe. Although samples were collected in areas at high risk of contamination, raw poultry meat and work surfaces reported a low level of presence of thermotolerant Campylobacter. The high percentage of C jejuni isolated from pigeon stools showed the importance of a continuous application of preventive measures by the food business operators and the surveillance activity by the Competent Authority.

Conflict of interests: the authors declare no potential conflict of interests.

Key words: Open-air market, Thermotolerant Campylobacter, Raw poultry meat, Pigeon stools.

Materials and Methods

A total of 280 samples were collected in 38 open-air markets in Turin, from September 2011 to December 2012. Samples (137 in 2011 and 143 in 2012) included poultry meat, swabs of surfaces where poultry is processed, stools of rock pigeon (Columba livia) and a dead pigeon. Meat samples and environmental swabs were collected at 86 counters in 30 market areas. In each sale counter a sample of poultry meat (84 chicken and 2 turkey meat samples) and a surface swab were collected. In
particular, poultry samples can be distinguished into breast (N=23), thigh with skin (N=41), wings with skin (N=1), whole chicken (N=1) and unknown (N=20); environmental swabs were performed on the working plane (N=64), on knife blade (N=19) and unknown (N=3). A 100 cm² of the working planes area was sampled, while on knife blades both sides of the entire surface were swabbed. Swab sampling was carried out by wiping back and forward the whole surface rapidly. The area was first swabbed with one side of the swab and then it was swapped again with the opposite side of the same swab. A total of 108 biological samples (107 stool and 1 dead pigeon) was collected in 36 market areas. Stool samples were collected in the period January-March (N=6), in the second (N=18), third (N=32) and fourth quarter (N=51). Sampling was carried out by veterinarians of the local public health service (ASL TO1). Samples were transported at refrigeration temperature to the Istituto Zooprofilattico Sperimentale Piemonte, Liguria and Valle d’Aosta (IZSPLV), where microbiological analyses were performed.

Environmental swabs and food samples were processed according to ISO 10272-1:2006 (ISO, 2006) method. Faecal samples and the dead pigeon animal were analysed according to ISO 10272-1:2006 method (OIE manual of diagnostic tests and vaccines for terrestrial animals). Identification of Campylobacter species was performed by biochemical system API Campy (API-bioMérieux SA, Marcy l’Etoile, France).

Results

Out of 280 samples, 33 (11.8%) resulted positive for thermophilic Campylobacter. In particular, among the 86 meat samples only two (2.3%) resulted positive for Campylobacter coli: one sample of chicken breast in 2011 and one sample of chicken thigh in 2012, both collected in November, in two different markets of Turin area (open-air market in Piazza Vittoria and in Piazza della Repubblica). All food contact surfaces were negative. Out of 107 faecal samples, 30 (28%) resulted positive for Campylobacter jejuni (12 samples in 2011 and 18 samples in 2012). C. jejuni subspecies jejuni (83.3%) was the most frequently subspecies isolated in stool samples, followed by C. jejuni subsp. doylei (16.7%). C. jejuni subsp. jejuni was isolated from the dead pigeon collected in 2011 (Table 1). Stool samples collected during the January-March period showed the highest level of contamination with 100% (6/6) positive followed by the October-December period with 30% (15/51); finally the second quarter with 28% (5/18) and the third with 12.5% (4/32). Considering stool samples, 52.7% (19/36) of sampled areas resulted positive for

<table>
<thead>
<tr>
<th></th>
<th>2011 Meat</th>
<th>Swabs</th>
<th>Stool</th>
<th>Dead pigeons</th>
<th>2012 Meat</th>
<th>Swabs</th>
<th>Stool</th>
<th>Dead pigeons</th>
</tr>
</thead>
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<tr>
<td>C. coli</td>
<td>1</td>
<td>0</td>
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<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>C. jejuni subsp. jejuni</td>
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<td>0</td>
<td>9</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>16</td>
<td>0</td>
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<tr>
<td>C. jejuni subsp. doylei</td>
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<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Negative</td>
<td>41</td>
<td>42</td>
<td>40</td>
<td>0</td>
<td>43</td>
<td>44</td>
<td>37</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>42</td>
<td>42</td>
<td>52</td>
<td>1</td>
<td>44</td>
<td>44</td>
<td>55</td>
<td>0</td>
</tr>
</tbody>
</table>

C. coli, Campylobacter coli; C. jejuni subsp. jejuni, Campylobacter jejuni subspecies jejuni; C. jejuni subsp. doylei, Campylobacter jejuni subspecies doylei.
thermophilic Campylobacter. Stool samples collected from open-air market of Piazza della Repubblica showed the highest percentage of positivity: 50% of samples (2/4) in 2011 and 90% (9/10) in 2012. Market areas where stool samples were collected are showed in Figure 1.

Discussion

High concentrations of animals in areas such as public gardens and market areas, could be a risk for citizens’ health: direct contact with infected animals or contaminated material thereof could represent a source of Campylobacter spp. contamination for humans (OIE, 2008).

This survey showed that 52.7% of the market areas, sampled for pigeon faecal material, resulted positive for Campylobacter spp.: the high prevalence of positive samples (28%) demonstrates how birds could be considered as vectors and reservoirs for Campylobacter spp. in urban environment. Even if only pigeon stools were collected, other urban species as well, such as sparrows, starlings and seagulls, should be considered a possible source of contamination. Moreover, it should not be underestimated the role of insects, such as flies, which may be a mechanical means of food contamination. Considering the characteristics of poultry meat as raw material and the working conditions, structural and management limits of market areas, these data demonstrate a low risk of contamination by thermophilic Campylobacter. On the contrary, the high prevalence of positive stool samples should be taken into account, because of the high density of pigeons in market areas in Turin. These birds could be considered a source of contamination for environment and food. This evidence suggests the need for correct and continuous application of preventive measures by the food business operators supported by the surveillance activities carried out by the Competent Authority.

The microbiological data reported in the present study show a low contamination level both in poultry meat and environmental swabs. Considering the characteristics of poultry meat as raw material and the working conditions, structural and management limits of market areas, these data demonstrate a low risk of contamination by thermophilic Campylobacter. On the contrary, the high prevalence of positive stool samples should be taken into account, because of the high density of pigeons in market areas in Turin. These birds could be considered a source of contamination for environment and food. This evidence suggests the need for correct and continuous application of preventive measures by the food business operators supported by the surveillance activities carried out by the Competent Authority.

Conclusions

The microbiological data reported in the present study show a low contamination level both in poultry meat and environmental swabs. Considering the characteristics of poultry meat as raw material and the working conditions, structural and management limits of market areas, these data demonstrate a low risk of contamination by thermophilic Campylobacter. On the contrary, the high prevalence of positive stool samples should be taken into account, because of the high density of pigeons in market areas in Turin. These birds could be considered a source of contamination for environment and food. This evidence suggests the need for correct and continuous application of preventive measures by the food business operators supported by the surveillance activities carried out by the Competent Authority.

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