CAMPYLOBACTERS ON PROCESSED BROILER CARCASSES
IN THE ANNUAL CYCLE

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Abstract

The work was focused on changes in the prevalence and
numbers of Campylobacter sp. on processed broiler carcasses
in Poland in the annual cycle. The subjects of the analysis were
skin samples collected monthly directly from the poultry
processing line, prior to the breast filleting production stage.
The procedure used included simultaneous pre-enrichment and
direct plate counting according to ISO 10272 (1995) with the
detection level (1 cfu g⁻¹), followed by biochemical
identification of the species. For the strains identified
biochemically as C. jejuni confirmation by the nested PCR
method was done. The incidence rate of campylobacter
contamination of processed broiler carcasses tested exceeded
88%, with numbers of Campylobacter sp. ranging from 10⁰ to
10⁴ cfu g⁻¹ and C. jejuni being the most frequently isolated
species. The differences in the incidence rate of campylobacter
contamination of the skin of the processed broiler carcasses
were statistically significant among the seasons and highly
statistically significant among months. As for the
campylobacter contamination level of processed poultry
carcasses, variations in the numbers of the bacteria were
statistically not significant among the seasons and highly
significant for months, being visibly lower in June and the
highest in July.

Key words: processed broiler carcasses, Campylobacter, food contamination, seasons.

Thermotolerant campylobacters - C. jejuni/C. coli in
particular - are one of the most frequent causes of food
borne diseases in humans worldwide (www.who.int/mediacentre/
factsheets/fs255/en) with numbers of people affected annually being much higher
than the documented cases (2, 3). Moreover, the food
borne cases and outbreaks recorded worldwide point out
to undercooked chicken and handling of poultry as
important risk factors of campylobacteriosis (9, 18)
particularly in summer (15).

Campylobacter-positive poultry, in general, show
no symptoms of the infection but can excrete high
numbers of the microorganisms from about 10² to 10⁷
per g of faeces (5, 26). When present, horizontal transfer
of campylobacters within the flock is a matter of days.
Until now, industrial slaughter and processing of
poultry, favour the contamination of the broiler
carcasses, initially free of campylobacters (11, 13, 17).

The data on the incidence rate and numbers of
campylobacters on poultry raw materials and products
differ considerably by the country and type of the
product (1, 20, 22, 23, 25). However, such data for
Poland are scarce (16).

The growing popularity of poultry, with the total
annual production of that type of meat in Poland
exceeding one million tons and consumption calculated
at 21.3 kg per capita annually (7) give rise to questions,
whether poultry pose a threat to consumers also in
Poland and the Poles are at risk of campylobacteriosis,
similarly to that in other European countries. That was
why the aim of the study was to estimate the incidence
rate and numbers of thermotolerant campylobacters on
the skin of processed broiler carcasses, at the poultry
processing plant level in Poland, in the annual cycle.

Material and Methods

Sampling. Bacteriological analysis was performed
on skin samples collected directly from broiler carcasses
at the processing line of one of the poultry processing
plant in Northern Poland. Once a month, 20 skin
samples were collected at random. Disposable gloves
were used to remove skin from a broiler carcass prior to
breast fillet production stage. Skin samples were
transferred directly to separate sterile stomacher bags
with filters, placed within the insulated cooling box at
~4°C when transported to the laboratory and subjected
to analysis within four hours. The weight of skin
samples ranged from 26.0 to 105.0 g. Between
December 2001 and December 2002, a total of 260 skin
samples were tested.

Confirmation of the presence of campylobacters
and their enumeration. The presence of Campylobacter sp. on skin samples and contamination
level were estimated using procedures including 24-48 h
pre-enrichment of initial dilution in Preston broth prior to isolation on modified Cefoperazone Charcoal Desoxycholate Agar (mCCDA) as well as by direct plate counting on mCCDA medium according to ISO 10272: 1995.

Initial (1:2) and serial 10-fold dilutions prepared in Buffered Peptone Water (ISO 10272: 1995) were spread on mCCDA medium in duplicate and incubated at 42°C under microaerophilic atmosphere for 48 h. Characteristically growing colonies were counted. Typically, growing colonies, selected at random, were tested for purity and subjected to primary identification according to ISO 10272: 1995.

As for the pre-enrichment step, the initial material (2 ml of 1:2 of initial dilution) was transferred into 10 ml of the Preston broth supplemented with sterile lysed defibrinated horse blood and antibiotic solution (SR 204E, Oxoid) and incubated for 24-48 h at 37°C prior to isolation on modified Cefoperazone Charcoal Desoxycholate Agar (mCCDA).

Typically, growing colonies were isolated at random and subjected to further identification steps only if the direct analysis gave negative result and/or types of colonies differed visibly. The detection level of the methods applied was 1 cfu g⁻¹.

**Confirmation tests.** Biochemical identification of the isolated strains was based on a simplified set of tests according to ISO 10272: 1995 and standard tests apiCAMPY (bioMerieux). The identification of all strains classified as *C. jejuni* was confirmed by the nested PCR method with two pairs of primers: C-1 + C-4 and C-1 + C-2 (6, 28). For genetic identification, the isolated strains were stored in AUX Medium (apiCAMPY - bioMerieux) supplemented with 10% glycerol (growth turbidity 4-6 in Mc Farland’s scale) at -20°C.

**Statistical analysis.** Statistical analyses based on the 1-factor variance analysis (ANOVA) and the chi-square (χ²) test (19) were used to compare the differences in the incidence rate and numbers of campylobacters on skin of processed broiler carcasses between seasons or months of the year. Differences in the isolation rate and numbers were considered significant when P ≤ 0.05.

**Results**

Thermophilic campylobacters were isolated from 88.1% (229/260) of broiler skin samples collected directly from the poultry processing line at the stage prior to breast fillet production.

Out of 229 campylobacter-positive samples, in 54.6% (n=125) their numbers ranged from 10² to 10⁴ cfu g⁻¹, followed by contamination level within the range of 10³-10⁴ cfu g⁻¹ in 29.7% (68/229) and exceeding 10⁴ cfu g⁻¹ only occasionally (Table 1). The percentage of campylobacter-positive samples, for the dominating range, was the highest in autumn (63.8%), while spread in numbers of *Campylobacter* sp. was the lowest one compared to other seasons (Fig.1). Differences in the incidence rate of campylobacters on skin samples among the seasons were statistically significant (P <0.01) while numbers of *Campylobacter* sp. per g of the carcass skin were not significant (Fig.1, Table 1).

Monthly fluctuations in the prevalence and numbers of campylobacters, expressed as mean log₁₀ cfu g⁻¹, are presented in Table 2 and in Fig.2. In eight out of thirteen experimental months, campylobacters were present in 100% of the samples tested with a visibly lower incidence rate noted for April and June (Table 2). With numbers of campylobacters oscillating between 0.74 log₁₀ cfu g⁻¹ (June) and 3.29 log₁₀ cfu g⁻¹ (July), fluctuations noted were sinusoid in nature (Fig.2). Yet the differences noted, both, in the incidence rate and numbers of campylobacters on skin among particular months in the annual cycle were statistically significant.

**Table 1**

Seasonal fluctuation in the prevalence and numbers of thermotolerant campylobacters on processed broiler carcasses in the annual cycle

<table>
<thead>
<tr>
<th>Cfu g⁻¹</th>
<th>Spring (n=60)⁹</th>
<th>Summer (n=60)</th>
<th>Autumn (n=60)</th>
<th>Winter (n=80)</th>
<th>Total (n=260)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - &lt;10¹⁰</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (1.4)</td>
<td>1 (0.4)</td>
</tr>
<tr>
<td>≥10¹ - &lt;10²</td>
<td>5 (10.4)</td>
<td>7 (14.3)</td>
<td>10 (17.2)</td>
<td>10 (13.5)</td>
<td>32 (14.0)</td>
</tr>
<tr>
<td>≥10² - &lt;10³</td>
<td>27 (56.3)</td>
<td>25 (51.0)</td>
<td>37 (63.8)</td>
<td>36 (48.6)</td>
<td>125 (54.6)</td>
</tr>
<tr>
<td>≥10³ - &lt;10⁴</td>
<td>15 (31.3)</td>
<td>17 (34.7)</td>
<td>11 (19.0)</td>
<td>25 (33.8)</td>
<td>68 (29.7)</td>
</tr>
<tr>
<td>&gt;10⁴</td>
<td>1 (2.0)</td>
<td>0</td>
<td>0</td>
<td>2 (2.7)</td>
<td>3 (1.3)</td>
</tr>
<tr>
<td></td>
<td>48 (80)</td>
<td>49 (81.7)</td>
<td>58 (96.7)</td>
<td>74 (92.5)</td>
<td>229 (88.1)</td>
</tr>
</tbody>
</table>

⁹ - positive results after prior enrichment;
⁹ - number of samples tested.
### Table 2
Monthly fluctuation in the prevalence and numbers of thermotolerant campylobacters on processed broiler carcasses in the annual cycle

<table>
<thead>
<tr>
<th>Month</th>
<th>Positive samples/number tested (%)</th>
<th>Mean log&lt;sub&gt;10&lt;/sub&gt; cfu g&lt;sup&gt;-1&lt;/sup&gt;</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>XII</td>
<td>20/20 (100)</td>
<td>2.55 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>14/20 (70)</td>
<td>1.37 ± 0.25</td>
<td>± 0.25</td>
</tr>
<tr>
<td>II</td>
<td>20/20 (100)</td>
<td>3.00 ± 0.13</td>
<td>± 0.13</td>
</tr>
<tr>
<td>III</td>
<td>20/20 (100)</td>
<td>2.76 ± 0.11</td>
<td>± 0.11</td>
</tr>
<tr>
<td>IV</td>
<td>8/20 (40)</td>
<td>0.80 ± 0.25</td>
<td>± 0.25</td>
</tr>
<tr>
<td>V</td>
<td>20/20 (100)</td>
<td>2.94 ± 0.14</td>
<td>± 0.14</td>
</tr>
<tr>
<td>VI</td>
<td>9/20 (45)</td>
<td>0.74 ± 0.21</td>
<td>± 0.21</td>
</tr>
<tr>
<td>VII</td>
<td>20/20 (100)</td>
<td>3.29 ± 0.09</td>
<td>± 0.09</td>
</tr>
<tr>
<td>VIII</td>
<td>20/20 (100)</td>
<td>2.67 ± 0.08</td>
<td>± 0.08</td>
</tr>
<tr>
<td>IX</td>
<td>19/20 (95)</td>
<td>2.27 ± 0.18</td>
<td>± 0.18</td>
</tr>
<tr>
<td>X</td>
<td>20/20 (100)</td>
<td>2.58 ± 0.08</td>
<td>± 0.08</td>
</tr>
<tr>
<td>XI</td>
<td>19/20 (95)</td>
<td>2.57 ± 0.18</td>
<td>± 0.18</td>
</tr>
<tr>
<td>XII</td>
<td>20/20 (100)</td>
<td>2.96 ± 0.12</td>
<td>± 0.12</td>
</tr>
</tbody>
</table>

**Fig. 1.** Seasonal changes in *Campylobacter* sp. numbers on skin of processed broiler carcasses.

**Fig. 2.** Monthly changes in *Campylobacter* sp. numbers on skin of processed broiler carcasses in the annual cycle.
Among 52 strains subjected to biochemical identification with the api-Campy tests, most (42/52) were classified as \textit{C. jejuni} (80.8\%) with the domination of \textit{C. jejuni jejuni} 1 (27/42) and \textit{C. jejuni doylei} (14/42) subspecies followed by \textit{C. coli} (7/42) constituting 13.5\% of the strains subjected to testing. The identification usually was either very good (27/52) or a good one (18/52 - Table 3).

Surprisingly, also strains with either doubtful or non-acceptable for \textit{C. jejuni} results of biochemical identification (4/42) were confirmed as \textit{C. jejuni} by the nested PCR method.

\section*{Discussion}

Results of surveys conducted at the poultry processing plant level (Table 1) indicated that the incidence rate of \textit{Campylobacter} sp. contamination of processed broilers’ skin ranged from 80\% (spring) to 96.7\% (autumn) among the seasons. Though the differences in the incidence rate of contamination with campylobacters among the seasons were statistically significant, the differences in their numbers (cfu g$^{-1}$) among the seasons were not significant (Fig. 1).

Surveys on the prevalence and numbers of \textit{Campylobacter} sp. on poultry products, conducted mostly at retail level, differ by type of the sample tested (whole carcass or cuts fresh or frozen), sampling method used (rinsing, excision, swabbing, homogenising), enumeration method applied (plating, MPN technique), and conversion units used (cfu g$^{-1}$, cfu carcass$^{-1}$, cfu cm$^{-2}$, cfu cm$^{-3}$) making the comparison of results difficult (10, 12, 16, 21, 22).

According to Rosenquist et al. (27), the season influenced the \textit{C. jejuni} detectability and the contamination rate of broiler carcasses at retail. Broiler carcasses collected from the local supermarkets in England were \textit{C. jejuni} positive in 69\% (229/330) with the highest recovery rates noted from May through October (93, 97, 97, 97, 87, and 93\%), and the lowest one in December (7\%) and January (33\%).

Monthly differences noted in the contamination rate of processed broilers’ skin with campylobacters at the plant level, though not so spectacular, were statistically significant (P≤0.05). Eight of 13 sets of the skin samples collected monthly were \textit{Campylobacter} positive in 100\% (Table 2). Campylobacter numbers, expressed as mean log$_{10}$ cfu g$^{-1}$, ranged from 0.74 to 3.29 being the highest in July. Samples collected in April and June were campylobacter-positive in 40-45\% and contamination level with campylobacters was the lowest one (Table 2, Fig. 2).

Similar numbers of campylobacters, ranging from 2 to 3 log$_{10}$ cfu g$^{-1}$ in the breast, thigh, and drumstick skin samples collected at retail, were noted by Berrang et al. (4). The contamination level of the neck skin was slightly higher. Depending on the type of the water-bath the carcasses were immersed in, campylobacter numbers ranged from 1.88 to 3.59 log$_{10}$ cfu g$^{-1}$ (10).

According to Rosenquist \textit{et al.} (20), there is a correlation between \textit{Campylobacter} numbers in the intestines and on carcasses at slaughter and their numbers on the neck skin being by 4.2 log lower. Yet, the numbers of thermotolerant campylobacters on the neck skin do not change visibly during operations following slaughter ranging from 1.43 to 3.24 log$_{10}$ g$^{-1}$ prior to packing of chilled or frozen chicken carcasses. According to Allen \textit{et al.}(1) and Slader \textit{et al.} (24), though prevalence of campylobacters on chicken can be high, the numbers of cross-contaminated carcasses are rather low ranging from 1.1 (24) to 2.5 log$_{10}$ cfu per carcass (1).

High prevalence of \textit{Campylobacter} sp. on processed broilers’ skin throughout the experimental year (Table 2) gives, rather, an evidence for easy cross-contamination of carcasses with campylobacters, when present on broilers subjected to slaughter. In an automated poultry processing line, steps such as stunning, de-feathering, evisceration, washing, and portioning may favour such cross-contamination. Except for the poultry processing stages, a humid air in the production premises may contribute to the contamination of broilers with campylobacters. The pilot scale experiment on the air quality, conducted at the same poultry processing plant, along the poultry processing line, proved air of some production premises to be contaminated with campylobacters. The contamination varied between the processing stages, exceeding 10$^{3}$ cfu per m$^{3}$ for rooms where stunning and killing, scalding, de-feathering, and evisceration took place (data not presented).

The less species diversity of campylobacters on the skin of processed broilers as compared to data dealing with bacterial species composition on poultry at retail was noted. The isolated strains were mostly \textit{C. jejuni} (80.8\%) - \textit{C. jejuni jejuni} 1 (27/42), and \textit{C. jejuni doylei} (14/42) in particular - followed by \textit{C. coli} (13.5\%). Three others were classified as \textit{C. hyointestinalis} (2) and \textit{C. foetus} (Table 3).

In swabs collected from the broiler carcasses by Kwiatek \textit{et al.} (16), \textit{C. jejuni}, \textit{C. coli}, and \textit{C. lari} constituted 59.1\%, 34.2, and 6.3\% of the isolated strains, respectively.

Of the 341 \textit{Campylobacter} strains isolated by Sallam (21) from various chicken meat and chicken by-products (wings, liver, gizzard, heart), 278 (81.5\%) were classified as \textit{C. jejuni}, with such identification confirmed by PCR in 99.4\% of the strains.

According to the above presented data, it seems obvious for \textit{C. jejuni} to be the \textit{Campylobacter} sp most frequently present on poultry meat, no matter the climatic zone or country.

The presented results make indirect evidence for broilers to pose a high risk of campylobacteriosis to consumers in Poland to the extent similar to the one in other European countries.

Increased numbers of campylobacteriosis cases during summer vacations, including those acquired abroad, noted in countries such as Sweden or Norway (8), confronted with the highest numbers of \textit{Campylobacter} sp. on processed broiler carcasses noted in July (own experiment) suggest there to be a higher risk for such outbreaks in July also in Poland.
Table 3
Campylobacter species isolated from processed broiler carcasses – identification based on apiCAMPY tests

<table>
<thead>
<tr>
<th>Species</th>
<th>No. of strains tested</th>
<th>Results of identification</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>exellent</td>
<td>very good</td>
</tr>
<tr>
<td>C. j. jejuni 1</td>
<td>27</td>
<td>-</td>
</tr>
<tr>
<td>C. j. jejuni 2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>C. j. doylei</td>
<td>14</td>
<td>-</td>
</tr>
<tr>
<td>C. jejuni*</td>
<td>42</td>
<td>1</td>
</tr>
<tr>
<td>C. coli</td>
<td>7</td>
<td>-</td>
</tr>
<tr>
<td>C. lari</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>C. upsaliensis</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>C. hyointesinalis</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>C. fetus fetus</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>C. sput. fecalis</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Σ</td>
<td>52</td>
<td>1</td>
</tr>
<tr>
<td>%</td>
<td>(1.9)</td>
<td>(52)</td>
</tr>
</tbody>
</table>

a – identification confirmed by the PCR method.

The risk increases with, usually seasonal, rush of people to different sites located e.g. by the sea-shore, with seasonally functioning gastronomy sites offering chicken based fast foods for tourists.

Different suppliers, growing production scale, and automated process coupled with wholesale trade of poultry to retailers favour cross-contamination of raw poultry meat with campylobacters posing a risk of campylobacteriosis to consumers. As to avoid the cross-contamination of poultry and to reduce the load of campylobacters on broilers, various preventive and hygienic measures have been suggested and applied, at both at farm and poultry processing plant level (13, 14, 20). However, the effects are far from the desirable ones. From the public health point of view, the high prevalence of C. jejuni noted on processed broilers in Poland makes it essential, for preventive measures to be undertaken also when handling poultry and processing it at home.

In conclusion, the incidence rate of campylobacters contamination of processed broilers in Poland is quite high. The numbers of campylobacters on broilers’ skin range mostly from 10^2 to 10^3 cfu g^-1 exceeding 10^4 only occasionally. The differences noted in the incidence rate of campylobacter contamination of processed broilers and, both, in the seasons and months of the year, as well as in the numbers of Campylobacter sp. were significant statistically. The highest numbers of campylobacters on processed broilers were noted in July. C. jejuni jejuni 1 and C. jejuni doylei were subspecies most frequently isolated from processed broilers.

References
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