Occurrence of *Campylobacter* on carcasses of slaughtered animals between 2009 and 2013

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Abstract

A total of 2668 swabs from poultry (n = 2166), pig (n = 311), and cattle (n = 191) carcasses were collected in slaughterhouses all over Poland and tested for the presence of *Campylobacter*. It was found that 1319 (49.4%) of them were contaminated with these bacteria. The percentages of the positive samples were different in each year of the study and the highest proportion of *Campylobacter* contaminated samples occurred in 2009, when 64.1% of investigated carcasses were positive. On the other hand, the lowest prevalence of *Campylobacter* was observed in 2013, in the last year of the survey. In all kind of carcass samples both *C. jejuni* and *C. coli* were identified, although the pork meat was more contaminated with *C. coli* (75.3% of positive samples) than with *C. jejuni* (24.7%), whereas poultry was nearly equally positive for *C. jejuni* and *C. coli* (50.6% and 49.4% respectively). The analysis of seasonal contamination of the carcasses revealed that more positive results were found during the second half of year than between January and June. The prevalence of *Campylobacter* showed that in all provinces, except one (Pomorskie), the mean percentage of the positive samples was above 40%. The most contaminated samples were identified in Lubelskie (69.3%) and Zachodniopomorskie (66.3%) regions. The obtained results showed that slaughtered animals in Poland, especially broilers, were often contaminated with *Campylobacter*, either *C. jejuni* or *C. coli*.

Keywords: carcasses, *Campylobacter* contamination, 5-year study, seasonal variation, Poland.

Introduction

Campylobacteriosis has been the most commonly reported foodborne zoonosis in humans in the European Union (EU) since 2005 (5). In 2012, the number of notified cases of *Campylobacter* infection was 214,268, and in average 55.49 confirmed campylobacteriosis cases per 100 000 EU inhabitants were reported. At the same time, 431 verified human campylobacteriosis cases were identified in Poland, with a low infectious rate (1.12 per 100 000), but this small number of infections may be due to an inadequate laboratory identification system (5). Poultry meat is the most important source of *Campylobacter* infection, especially with *C. jejuni* (5, 6, 8, 10, 18). It is estimated that 50% to 80% of human campylobacteriosis cases have been attributed to the chicken (broiler) reservoir (5). Other epidemiological evidences also indicate the major role of zoonotic transmission of *Campylobacter* from poultry to humans (4, 10, 11). Broiler meat is usually contaminated during slaughter or during the dressing or trimming processes. The source of the bacteria may be the chickens or other *Campylobacter* contaminated carcasses or equipment (10, 19). There are several evidences that in some countries *Campylobacter* prevalence in broiler flocks increases during warmer months of the year, therefore, it is likely that more poultry meat gets contaminated during these periods compared with months with lower prevalence in broilers. According to EFSA data for 2012, 23.6% of broiler meat samples (single or batch) were found to be positive for *Campylobacter* in the European Union Member States, ranging from 0% in the Czech Republic up to 80.6% in Luxembourg (5).

Most studies on the prevalence of *Campylobacter* focus on poultry and poultry meat; however, pigs and cattle are also considered as potential reservoir of these pathogenic bacteria (1-3, 12-15, 17). Therefore, it is
crucial to investigate the prevalence of *Campylobacter* on the carcasses of these food producing animals to assess a potential risk for consumers.

The aim of the study was to determine the occurrence of *Campylobacter* in broiler, pig, and cattle carcasses at slaughterhouse level in Poland during a 5-year period as well as to investigate an influence of season on their contamination.

### Material and Methods

**Sample collection.** Since July 2009 to December 2013, 2668 swab samples collected from poultry (broilers) (n = 2166), pig (n = 311), and cattle (n = 191) carcasses were examined for the presence of *Campylobacter* sp. The samples were randomly collected in all provinces (administrative districts) of Poland at slaughterhouses. In the case of poultry carcasses, the neck skin and skin surface under the wings were wiped 10 times with sterile swabs directly after immersion chilling (0 to 4°C) but before further processing, such as freezing, cutting, or packaging. The swabs were immediately transported to a laboratory in Amies transport medium (Medlab, Poland). Pig and cattle carcasses were swabbed with sponges from the brisket area (100 cm²) after exsanguination of the animals. The sterile sponges were rubbed 10 times vertically and 10 times in a horizontal direction, and then were placed in autoclavable stomacher bags, pre-moistened with 10 mL of Maximum Recovery Diluent (MRD, Oxoid, UK; 0.1% peptone, 0.85% NaCl). The swabs were then placed in a cooler box and immediately transported to the laboratory.

**Bacterial isolation and identification.** In the laboratory, the poultry swabs were put into 5 mL of Bolton broth plus 5% leaked horse blood and modified Bolton broth – selective supplement containing antimicrobials (vancomycin, cefoperazone, trimethoprim, amphotericin B) to prevent non-target microbials (Oxoid, UK). The cultures were incubated at 41.5°C for 48 h under microaerobic conditions using the CampyGen kit (Oxoid). *Campylobacter* were isolated and identified according to the ISO 10272-1:2006 standard. Briefly, after the enrichment step, the cultures from swab samples were plated onto Karmali agar (Oxoid) and *Campylobacter* blood-free agar (Oxoid) with CCDA selective supplement (Oxoid) and incubated at 41.5°C for 48 h under microaerobic conditions. The plates were then examined for morphologically typical *Campylobacter* colonies and from each sample, one presumptive *Campylobacter* isolate was confirmed by PCR assay as previously described (20). Furthermore, the isolated strains were identified as *C. jejuni* or *C. coli* by PCR (20, 22).

In the case of cattle and pig carcasses, all four sponges used for swabbing were placed together into 200 mL of MRD and stomached for 3 min. After centrifugation at 1000 g for 15 min at 5°C, pellets were resuspended in 100 mL of selective enrichment Bolton broth. The enriched cultures were grown for 48 h at 41.5°C under microaerobic conditions and then plated onto Karmali agar (Oxoid) and *Campylobacter* blood free agar (Oxoid) with CCDA-selective supplement (Oxoid) followed by reincubation under the previously described conditions for 48 h. The isolated bacterial colonies were identified as *Campylobacter* as described for poultry samples.

**Reference strains.** Two *Campylobacter* reference strains were included in the study: *C. jejuni* ATCC 33560 and *C. coli* ATCC 43478.

### Results

During the study, a total of 2668 samples were collected at the slaughterhouse level and tested for the presence of *Campylobacter*. It was found that in 1319 (49.4%) out of them, especially poultry carcasses (1210; 55.9%), were contaminated with these bacteria (Table 1). The percentage of the positive samples varied in each year of the study, and the highest proportion of *Campylobacter* positive samples occurred in 2009, when 64.1% of investigated carcasses were contaminated with these bacteria. On the other hand, the lowest prevalence of *Campylobacter* was observed in the last year of the survey (2013); however, poultry samples were positive at the similar level as in the remaining periods. In all kinds of carcass samples, *C. jejuni* and *C. coli* were identified, although the pork was more contaminated with *C. coli* (75.3% of positive samples) than with *C. jejuni* (24.7%). However, the number of these samples tested was much lower as compared to those isolated from poultry (Table 1).

The seasonal contamination of the meat samples with *Campylobacter* is shown in Table 2. The high prevalence of the bacteria was identified all year round; however, an average more positive results were found in the second half of the investigated years than between January and June.

The analysis of the prevalence of *Campylobacter* revealed that in all provinces, except one (Pomorskie), the mean percentage of the positive samples was above 40% (Fig. 1). The most contaminated carcasses were identified in Lubelskie (69.3%) and Zachodniopomorskie (66.3%) regions. The identification of *Campylobacter* at the species level by PCR showed that *C. jejuni* was the most prevalent in Zachodniopomorskie province (78.7% of the positive samples), followed by Podlaskie (63.0%), Śląskie (61.4%), and Opolskie (58.1%). In the remaining regions of Poland, the carcasses were more commonly contaminated with *C. coli* than *C. jejuni*, especially in Lubuskie and Lubelskie provinces - 87.5% and 63.9% respectively.
### Table 1. Prevalence of *Campylobacter* in different carcass samples at slaughterhouse level during 2009-2013

<table>
<thead>
<tr>
<th>Year of sampling</th>
<th>Poultry</th>
<th>Pig</th>
<th>Cattle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tested</td>
<td>Positive (%)</td>
<td>Tested</td>
</tr>
<tr>
<td></td>
<td>Campylobacter sp.</td>
<td>C. jejuni</td>
<td>C. coli</td>
</tr>
<tr>
<td>2009</td>
<td>399</td>
<td>283 (70.9)</td>
<td>154 (54.4)</td>
</tr>
<tr>
<td>2010</td>
<td>476</td>
<td>276 (58.0)</td>
<td>154 (55.8)</td>
</tr>
<tr>
<td>2011</td>
<td>431</td>
<td>257 (55.0)</td>
<td>115 (48.5)</td>
</tr>
<tr>
<td>2012</td>
<td>429</td>
<td>245 (57.1)</td>
<td>109 (44.5)</td>
</tr>
<tr>
<td>2013</td>
<td>431</td>
<td>169 (39.2)</td>
<td>80 (47.3)</td>
</tr>
<tr>
<td>Total</td>
<td>2166</td>
<td>1210 (55.9)</td>
<td>612 (50.6)</td>
</tr>
</tbody>
</table>

1 Since the number of positive samples in each year was below 10, the percentage was not calculated.

### Table 2. Prevalence of *Campylobacter* in poultry, pig, and cattle carcasses at slaughterhouse level during 2009-2013

<table>
<thead>
<tr>
<th>Year of sampling</th>
<th>Number of C. jejuni and C. coli positive samples/ total number of samples (% positive)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>January - March</td>
</tr>
<tr>
<td>2009</td>
<td>ND¹</td>
</tr>
<tr>
<td>2010</td>
<td>60 (42 C. j. + 18 C. c.)/131 (45.8)</td>
</tr>
<tr>
<td>2011</td>
<td>52 (19 C. j. + 33 C. c.)/109 (47.7)</td>
</tr>
<tr>
<td>2012</td>
<td>49 (21 C. j. + 28 C. c.)/96 (51.0)</td>
</tr>
<tr>
<td>2013</td>
<td>44 (20 C. j. + 24 C. c.)/100 (44.0)</td>
</tr>
<tr>
<td>Total</td>
<td>205 (102 C. j. + 103 C. c.)/436 (47.0)</td>
</tr>
</tbody>
</table>

¹ No data, ² C. j. - C. jejuni, ³ C. c. - C. coli
Discussion

The long-term study demonstrated that the carcasses of meat producing animals slaughtered in Poland are often contaminated with Campylobacter. It is not a surprise in the case of broilers, since this kind of meat has been found to be Campylobacter-positive in many countries (4, 6, 8, 11, 12, 14, 19). However, in the present investigation pig and cattle carcasses were also highly contaminated with these bacteria, although a low number of samples tested might have an influence on the obtained results. The percentage of positive results over the study period was rather similar in 2010, 2011, and 2012, whereas more carcasses were contaminated in the first year of the survey but it may be due to a lower number of samples tested. On the other hand, the lowest prevalence of Campylobacter was noted in 2013, when only 33.9% of the investigated samples contained these bacteria.

The prevalence of Campylobacter on chicken carcasses at the slaughterhouse level varies among European countries but usually is high (5, 6). The results of the European Union-wide baseline survey on Campylobacter on broiler carcasses conducted in 2008 showed that at Community level, 75.8% of samples were contaminated with these bacteria (6). The prevalence of Campylobacter was from 4.9% in Estonia and 5.5% in Finland to 94.3% in Malta and 98.3% in Ireland. At the same time, 80.4% of broiler carcasses were positive for Campylobacter in Poland. The results of the present study, which covers a 5-year period, demonstrated that less than 50% of similar chicken carcass samples were contaminated. There is no such earlier data concerning pig and cattle samples, thus, it is not possible to compare the present results. However, the studies performed in other countries showed that such food producing animals at the slaughterhouse level were also often positive for Campylobacter, although the percentage of such samples was lower than identified during the present survey, and 81 (26.0%) samples out of 311 tested were positive (2, 8, 19). Biasi et al. (2) demonstrated that 18.9% of pig carcasses were Campylobacter-positive (37 samples tested), whereas in Belgium 17% of 383 similar samples were contaminated (8). Similar results were obtained in the Czech Republic (18% of pig carcasses tested in 2001) (19). On the other hand, in the study performed by Quintana-Hayashi et al. (16), 27.9% of pig carcasses were positive for Campylobacter, whereas in Canada only 8.8% of such meat was contaminated with these bacteria (3).

As shown in the present study, 14.7% (28 out of 191 samples tested) of bovine carcasses were contaminated with Campylobacter. There are rather few similar investigations performed in other countries. In a survey in Belgium, 3.3% of beef carcass samples (n = 60) were positive for this pathogen (8), whereas in Italy 2% of raw beef meat (n = 151) was contaminated with Campylobacter (16). Furthermore, prevalence of Campylobacter was assessed in cattle in Finland (34.0% positive farms), in USA (43.3% and 51.2% positive faecal samples), and in Italy (43.7% rectal swabs) but all these results should not be compared with the contamination of cattle carcasses (1, 3, 7, 9).

The analysis of seasonal prevalence of Campylobacter in the carcasses revealed that during the
study period there were no marked differences in summer and winter months. In total, approximately the same percentage of samples was positive for the bacteria during July-September and October-December. Similar observations were done for January-March and April-June, although some difference between years could be found. These findings are opposite to the observations of other authors who noted a clear seasonal peak in the prevalence of Campylobacter in food producing animals, mainly poultry, in warmer months of the year (4, 11, 13). The previous results obtained during the EU baseline survey on Campylobacter conducted in 2008 also did not demonstrate any seasonal variation in the prevalence of the bacteria in broiler carcasses in Poland (6).

The results of the present study showed that Campylobacter in carcasses of the slaughtered animals was present all over Poland. However, in six regions the percentage of the positive results was over the mean value, especially in Lubelskie and Zachodniopomorskie provinces. In the case of Lubelskie, the EU baseline survey in 2008 also demonstrated the highest level of contamination of broiler carcasses all over country (6). It is difficult to explain why this region of Poland produces meat that is so highly contaminated with Campylobacter.

The prevalence of C. jejuni, which is more frequently associated with human campylobacteriosis than C. coli, was higher in several regions of Poland, especially in Zachodniopomorskie, Podlaskie, and Śląskie provinces. Similar observation in the case of Śląskie but not in the remaining two provinces was noted during the EU baseline study in 2008. However, at the EU level contamination of broiler carcasses with C. jejuni was much more frequent than with C. coli (67.9% and 39.4% respectively) (6). On the other hand, the geographical distribution of Campylobacter species obtained during the present study includes broiler, pig, and cattle samples. However, when only poultry carcasses were evaluated, the prevalence of C. jejuni and C. coli was almost equal (50.6% and 49.4% respectively).

In conclusion, it was shown that during the last 5 years slaughtered animals all over Poland, especially broilers, were often contaminated with Campylobacter, either C. jejuni or C. coli. The prevalence of the bacteria in the carcasses was noted irrespective of the season. Thus, a further Campylobacter monitoring and control programmes are needed in order to reduce the bacterial contamination level in food production chain.

References

5. EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control): The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2012. EFSA J 2014, 12, 3547.

