Risk Factors for the Presence of Campylobacter Sp. in Lithuanian Broiler Flocks

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Abstract: The objective of this study was to define the incidence of Campylobacter in Lithuanian broiler flocks. The incidence of both Campylobacter and Salmonella and the seasonal fluctuations on the occurrence of pathogens were focused in this study. Faeces, dust and water samples were obtained at the farms 1-2 days before the broiler slaughtering. The cecum was removed after slaughtering. Microbiological study of the faeces and cecum content showed, that 18.4% (95% CI 7.0-29.0) of flocks were colonized with Campylobacter sp. However, dust and water samples were found to be free of Campylobacter. The study of influence of other pathogens (Salmonella) on the prevalence of Campylobacter sp. showed, that 12.2% (95% CI 3.0-21.0) of broiler flocks were colonized with both pathogens (Campylobacter and Salmonella). Campylobacter jejuni was predominant among the Campylobacter-positive flocks. The majority of broiler flocks harbored Campylobacter in spring (30.7%).

Key words: Campylobacter, Salmonella, broiler flocks, seasonal fluctuations

INTRODUCTION
Campylobacteriosis is one of the most commonly reported bacterial foodborne infections worldwide (Allos, 2001). Campylobacter sp. are recognized as a major cause of human gastroenteritis in developed countries (Blaser, 1997). Consumption of undercooked poultry has often been identified as a risk for human campylobacteriosis (Kapperud et al., 1992; Pearson et al., 2000). Trying to avoid the Campylobacter contamination of poultry products should reduce the risk of human infection. Over the last decade, the occurrence and spread of Campylobacter in broiler flocks has been intensively studied in several countries. Flock prevalence with Campylobacter ranging from 18-90% had been reported in Europe (Evans and Sayers, 2000; Refregier-Petton et al., 2001; Newell and Fearnley, 2003). There are two possible routes of its transmission in poultry: horizontal and vertical. Horizontal transmission is believed to be mainly through contaminated water, litter, insects, wild birds, rodents, faecal contact or transferred by farm personnel via their boots (Evans and Sayers, 2000). Once Campylobacter enters a flock, all the chickens in the flock become colonized and stay colonized till the slaughter time (Lindblom et al., 1986). Various factors influencing the prevalence of Campylobacter in broiler flocks have been described in several studies. The main factors associated with an increased colonization of Campylobacter are the lack of hygiene barriers (Kapperud et al., 1993; Evans and Sayers, 2000); the presence of other domestic animals in poultry farms (Kapperud et al., 1993; Bouwknegt et al., 2004); several poultry-houses on the farm (Refregier-Petton et al., 2001; Bouwknegt et al., 2004); and the season of the year (Kapperud et al., 1993; Wallance et al., 1997; Obiri-Danso and Jones, 2000; Refregier-Petton et al., 2001; Sari et al., 2004). Conversely, broiler flocks that tested negative at the farm for Campylobacter were also negative after slaughter (Aho and Him, 1988). However, transporting broilers to the processing plant was shown to increase the prevalence of Campylobacter positive birds because of faecal contamination of skin and feathers by neighbouring birds during shipping (Stern et al., 1995). Many studies have showed the prevalence of broiler flocks with Campylobacter or Salmonella. However only few studies have investigated the possible association between the occurrences of both: Campylobacter and Salmonella. Though no association was found in Danish and Belgian studies (Wedderkopp et al., 2001; Rasschaert et al., 2007), a possible correlation was reported in Dutch poultry flocks (Jacobs-Reitsma et al., 1994; Jacobs-Reitsma et al., 1995). Consumers and farmers have been increasingly interested in organic food products. However, organic meat production involves potentially higher microbiological safety risk due to raising animals outdoors, the use of slow-growing breed, the prohibition to use antimicrobial preparations and exploiting of very small slaughtering facilities (Engvall, 2002). However, little is know about the microbiological status of conventional animal products in different countries. The objective of this study was to define the incidence of Campylobacter in Lithuanian conventional broiler flocks. We focused this investigation on a possible association
between the incidence of both *Campylobacter* and *Salmonella* depending on the season of the year.

**MATERIALS AND METHODS**

From August 2005 until April 2007 forty-nine conventional flocks from 4 different Lithuanian broiler farms were sampled. All conventional farms reared Cobb broilers. Faeces, dust and water samples were obtained at the farm from broiler flocks 1-2 days before the slaughtering. Five pooled samples were taken from each flock. The cecum from broiler flocks was removed after slaughter and kept on ice for the determination of *Campylobacter*. The samples were divided into two parts: one was placed into semisolid enrichment media for *Campylobacter* culture and the other for *Salmonella* culture. The samples for the study of *Campylobacter* were analyzed according to ISO 10272 using mCCDA and Karmali agar for plating and Preston Broth (Oxoid) for enrichment. The qualitative analysis was performed by enrichment of 1g of cecum content or faeces in 9 g of Preston broth (Oxoid) for 48 h at 42°C in a micro-anaerobic atmosphere (85% N₂, 10% CO₂, 5%O₂). The enrichment cultures were streaked onto mCCDA and Karmali plates and later were micro-anaerobically incubated for 48 h at 42°C. Colonies were confirmed by testing for motility and cell morphology, testing for catalase and oxidase activity. Confirmed *Campylobacter* isolates were biochemically differentiated with the API Campy test (BioMérieux). Water samples were filtered on a sterile 0, 45 µm microporous filter prior to adding the remaining broth. After enrichment, the samples were streaked onto selective agar media (Karmali agar) and plates were micro-anaerobically incubated for 48 h at 42°C. Colonies were confirmed by contrast microscopy and identified biochemically with API Campy test (BioMérieux).

*Salmonella* was isolated according to standard methods (International Organization for Standardization 6579, 1998). Twenty five gram samples of faeces, caecal and dust were homogenized with 225 mL of pre-enrichment medium buffered peptone water (BBL, Le Pont de Claix and France) and incubated for 18 h at 37°C; 25 mL of water sample were filled into a bulb followed by adding 225 mL of BBL and incubated for 18 h at 37°C. The pre-enriched culture (0.1 and 1 mL, respectively) was transferred to Rappaport-Vassiliadis (Oxoid, UK) broth and Selenite broth (Merck, Germany) and incubated for 24 h at 42°C. Following incubations, a loopful from each broth was streaked into XLD full Agar (Oxoid), Brilliant Green Agar (BBL, France), Hectoent Enteric Agar (Merck, Germany), or Rambach Agar (Merck) plates and incubated at 37°C for 24 h. The suspected *Salmonella* colonies were transferred into Klinger Agar (Oxoid CM33) and Urea Agar Base (Oxoid CM53) tubes. Following another overnight incubation at 37°C the *Salmonella* cultures were further identified biochemically, using API 20E system (bio Mérieux, France) and agglutination test using specific O and H antisera (Siifin, Germany, Murex, France and Seiken, Japan).

The 95% Confidence Intervals (CI) for the observed prevalence of *Campylobacter*-positive and *Salmonella*-positive samples were estimated by linear interpolation formula:

$$CI = p - z[(p(1-p))/n]^{0.5}, \ CI = p + z[(p(1-p))/n]^{0.5}$$

where:

- \( p \) = Number of positive samples/number of tested samples.
- \( z \) = (95%) 1.96.
- \( n \) = Number of tested samples.

**RESULTS**

The results of our investigations showed, that *Campylobacter* sp. colonized differently broiler flocks in separate farms and tested samples. From 4 farms studied *Campylobacter* sp. was detected only in two of them: C and D (Table 1). In farm D according to the results of investigation of ceca samples, 5 flocks from 15 were positive for *Campylobacter* sp. (33.3%). The study of the faeces samples showed, that 3 flocks from 7 were infected with *Campylobacter* (42.9%). In farm C according to the results of cecum samples only one flock was colonized with *Campylobacter*. The results of the cecum and faeces samples showed, that 22.2% of cecum samples and 25.0% of faeces samples were colonized with *Campylobacter*. All samples from dust and water samples proved to be *Campylobacter* negative. Totally, 9 flocks from 49 (18.4%) flocks were colonized with *Campylobacter* sp. The species distribution among Campylobacter-positive flocks showed that *Campylobacter jejuni* was predominant. From 9 positive flocks for *Campylobacter* sp. 8 were colonized with *Campylobacter jejuni* (88.9%) and only one flock with *Campylobacter coli* (11.1%).

The study of the influence of other pathogens on the prevalence of *Campylobacter* sp. showed, that 9 broiler flocks from 49 (18.4%) were colonized with *Campylobacter*, whereas *Salmonella* was isolated from 12 flocks (24.4%) (Table 2). Six flocks were positive for both pathogens *Campylobacter* and *Salmonella* (12.2%). Considering the seasonal variability of *Salmonella* and *Campylobacter* colonized flocks, the study period was divided into four periods according to the four seasons of the year (January-March, April-June, July-September and October-December).

The majority of *Campylobacter* positive broiler flocks were found in spring (30.7%) (Table 3). The percentage of *Campylobacter* infected broiler flocks was less in winter and summer (23.0 and 20.0%, accordingly).
Table 1: The results of tested flocks for Campylobacter sp. in different farms

<table>
<thead>
<tr>
<th>Farm</th>
<th>No. of houses</th>
<th>No. of tested flocks</th>
<th>No. of positive flocks (%)</th>
<th>No. of tested flocks</th>
<th>No. of positive flocks (%)</th>
<th>No. of tested flocks</th>
<th>No. of positive flocks (%)</th>
<th>No. of tested flocks</th>
<th>No. of positive flocks (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>4</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>B</td>
<td>6</td>
<td>5</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>C</td>
<td>50</td>
<td>4</td>
<td>1/25.0%</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>D</td>
<td>27</td>
<td>15</td>
<td>5/33.3%</td>
<td>7</td>
<td>3/42.5%</td>
<td>4</td>
<td>0</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>48</td>
<td>6/12.2%</td>
<td>12</td>
<td>5</td>
<td>3/25.0%</td>
<td>5</td>
<td>0</td>
<td>5</td>
<td>0</td>
</tr>
</tbody>
</table>

NT—not tested

Table 2: The incidence of Campylobacter, Salmonella sp. and Campylobacter + Salmonella in tested broiler flocks

<table>
<thead>
<tr>
<th>No. of tested flocks for Campylobacter (n = 49)</th>
<th>Positive (%)</th>
<th>CI (95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>9 (18.4)</td>
<td>7.3-28.7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>No. of tested flocks for Salmonella (n = 49)</th>
<th>Positive (%)</th>
<th>CI (95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12 (24.4)</td>
<td>12.1-35.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>No. of the tested flocks for Campylobacter + Salmonella (n = 49)</th>
<th>Positive (%)</th>
<th>CI (95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 (12.2)</td>
<td>3.0-21.0</td>
</tr>
</tbody>
</table>

similar seasonal influence for the prevalence of Salmonella was also detected. The majority of Salmonella positive broiler flocks was established in spring (54.0%). The incidence of Salmonella in winter, summer and autumn was less (30.0, 10.0 and 14.3%, accordingly).

**DISCUSSION**

Limited data exist on the prevalence of Campylobacter infection in conventional poultry flocks in different countries. According to the literature, in Europe this prevalence varies from 18 to >90%; in the northern countries it was found to be less than in southern European countries (Newell and Fearon, 2003). In the Netherlands about 30% of broiler flocks were contaminated with Campylobacter (Bouwknecht et al., 2004), while in Belgium 73% (Rasschaert et al., 2007). However, the prevalence of Campylobacter showed higher rates in mid and southern Europe, where up to 91% of positive flocks were found (e.g. in Italy) (EFSA, 2006). According to our results 18.4% of Lithuanian broiler flocks were found to be colonized with Campylobacter sp. A possible explanation for our finding of relatively low presence of Campylobacter in broiler flocks is the conventional production system used which is the most established housing type in Lithuania. The incidence of Campylobacter-positive flocks is generally higher (up to 100%) in organic and free-range flock farms (Berndtson et al., 1998; Heuer et al., 2001; Rodenburg et al., 2004) as compared to intensively reared ones.

The main reservoir of Campylobacter jejuni in poultry is the cecum (Rudi et al., 2004). Our results showed that cecum and faeces were colonized with Campylobacter, however dust and water samples proved to be Campylobacter negative. Seleha (2004) failed to isolate Campylobacter from swab samples of the walls, floors and dust from Malaysian chicken houses. There is an assumption that Campylobacter cannot survive for long period within the dehydrating conditions of dust. Bull (2006) suggest, that drinking water can be contaminated by faecal droppings during the rearing period and can serve as a transmission route. However the data from our investigations showed, that water was free of Campylobacter and could not be the risk factor for Campylobacter infection.

The species distribution among the Campylobacter-positive flocks showed that Campylobacter jejuni was predominant in conventional broiler flocks (88.9%) in Lithuania. These results are in agreement with those of other authors (Bouwknecht et al., 2004, Oyarzabal et al., 2005).

Although several risk factors for infection of broilers with Campylobacter sp. have been identified, knowledge about the various routes by which flocks become infected and their relative influence is still incomplete. One of the risk factors for the prevalence of Campylobacters in broiler flocks is the presence of >2 broiler houses on the farm (Refregier-Petton et al., 2001; Guerin et al., 2007). Our study showed that in farm (D), where the number of houses was 27, the prevalence of broiler flocks colonized by Campylobacter was 33.3% while in two other farms (A and B), wherein were only 4 and 6 houses, broiler flocks were found to be negative for Campylobacter. Several houses on the same farm may lead to an increased risk of Campylobacter through an intensive movement of farm workers between the houses. Dutch authors (Bouwknecht et al., 2004) suggest that animals on farms within 1 km have the highest impact on Campylobacter presence in Dutch broiler flocks.

There is only limited data on mixed infections on broiler flocks with Campylobacter and Salmonella (Jacobs-Reitsma et al., 1994, Wedderkopp et al., 2001; Rodenburg et al., 2004; Cui et al., 2005). In the study of Cui et al. (2005) the majority of conventionally bred chickens were contaminated with Campylobacter (74%) and only 44% with Salmonella. Other investigators also noted that 13% of organic broiler flocks were positive for Salmonella and 35% for Campylobacter (Rodenburg et
### Table 3. Distribution of Campylobacter sp. and Salmonella spp. according to the season of the year

<table>
<thead>
<tr>
<th>Season of the year</th>
<th>Winter</th>
<th>Spring</th>
<th>Summer</th>
<th>Autumn</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of tested flocks for Campylobacter</td>
<td>13</td>
<td>13</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>No. of positive flocks for Campylobacter (%)</td>
<td>3 (23.0%)</td>
<td>4 (30.7%)</td>
<td>2 (20.0%)</td>
<td>0</td>
</tr>
<tr>
<td>No. of tested flocks for Salmonella</td>
<td>27</td>
<td>13</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>No. of positive flocks for Salmonella (%)</td>
<td>7 (30.0%)</td>
<td>7 (54.0%)</td>
<td>1 (10.0%)</td>
<td>1 (14.3%)</td>
</tr>
</tbody>
</table>

However, in our study the number of Salmonella-positive flocks was bigger (24.4%) than that of Campylobacter infected ones (18.4%). Six flocks were positive for both pathogens Campylobacter and Salmonella (12.2%). It is difficult to explain which of these pathogens is “primary” or “secondary”. Further studies are needed to determine mixed infections in broiler flocks.

Seasonal variations in the occurrence of Campylobacter in broiler chickens have been described in several reports (Heuer et al., 2001; Wedderkopp et al., 2001; Huneau-Salauin et al., 2007). Although the seasonal aspect of broiler colonization by Campylobacter is often reported, the reason thereof is still unknown. In the study of Heuer et al. (2001), the highest prevalence of colonization was found from May to October. The risk of a flock being colonized with Campylobacter was higher in spring/summer period as compared to winter (Kovats et al., 2004; Huneau-Salauin et al., 2007). Other investigators (Wedderkopp et al., 2001) noticed that the majority of Campylobacter positive cloacal swabs were found in July, August and September while the lowest number of positive samples was found from January to April. The Danish scientists (Hald et al., 2004) suggest that flies may be an important source of Campylobacter infection of broiler flocks in summer. Our results showed that the risk of Campylobacter excretion by broiler chickens was increased in spring period. The geographical variation in the timing of the seasonal peak suggests that climate may be a contributing factor to Campylobacter transmission. On the other hand, the climate in Lithuania is very variable and changes from year to year. Taking into account the data of seasonal scattering in the occurrence of Campylobacter, we postulate, that warm period in comparing to cold one is more favourable to spreading Campylobacter infection in broiler flocks. The strong seasonal effect certainly made it more difficult to highlight other risk factors of bacteria colonization in our study.

**Conclusion:** In conclusion, no significant correlation between occurrence of Campylobacter and Salmonella infections in Lithuanian broilers was found, however the incidence of campylobacteriosis salmonellosis infection was associated with the season of the year.

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