Prevalence and counts of Campylobacter spp. in poultry meat at retail level in Estonia

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ABSTRACT

Campylobacter contamination of poultry meat at retail level was studied in two surveys during the twelve-month period of 2012 in Estonia. The data from these surveys were combined and analyzed, partially together, in order to comprehensively estimate the prevalence and possible seasonality of Campylobacter in poultry and in poultry meat products in Estonia. Mostly Estonian, Lithuanian and Latvian products, representing the most typical origins of poultry products on the Estonian retail market, were sampled and analyzed in these surveys. The first survey, organized by the Estonian Veterinary and Food Board, focused on Campylobacter prevalence in poultry meat at retail level. The second survey, at the Estonian University of Life Sciences, focused on Campylobacter prevalence and counts in fresh broiler chicken meat at retail level. Additionally, broiler chicken caecal samples were collected at slaughterhouse level for the estimation of the seasonal variation of Campylobacter colonization. Caecal samples were collected weekly from a broiler chicken slaughterhouse belonging to a company representing over 95% of all commercial broiler production in Estonia. A total of 606 poultry meat samples at retail level and 380 broiler chicken caecal samples at slaughterhouse level were collected and analyzed. A total of 20.8% of the poultry meat and 39.2% of the caecal samples were found positive for Campylobacter spp. The mean number of Campylobacters in fresh broiler chicken meat in the positive samples was 3.20 log10CFU/g. A distinct seasonal variation in the Campylobacter contamination of broiler chicken meat was observed, which peaked during the warm summer period.

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1. Introduction

Campylobacteriosis, caused by thermotolerant Campylobacter species, was the most commonly reported zoonosis in the European Union (EU) in 2012, with 214,268 confirmed human cases, an average of 55.49 confirmed cases of campylobacteriosis per 100,000 of the EU population (EFSA, 2014). According to EFSA scientific opinion (2011a), the actual number of all cases is estimated to be around nine million each year, and the total cost of
campylobacteriosis to public health in the EU is estimated to be around 2.4 billion euros a year (EFSA, 2011a). *Campylobacter* spp., predominantly *Campylobacter jejuni* and *Campylobacter coli*, most commonly cause gastroenteritis in humans, but also other extra-intestinal diseases and rare cases of post-infection complications, such as Miller-Fisher and Guillain–Barré syndrome, which can cause serious health complications (Fica et al., 2011; Kuwabara, 2011). Campylobacters may colonize the intestines of clinically healthy birds, and therefore the poultry products. Broiler chicken meat is considered to be the main source of foodborne *Campylobacter* infection (EFSA, 2013; FAO/WHO, 2001; Humphrey, O’Brien, & Madsen, 2007). In its scientific opinions, EFSA has concluded that the handling, preparation and consumption of broiler meat may account for 20%–30% of all human campylobacteriosis cases, while 50%–80% may be attributed to the chicken reservoir as a whole. Therefore, the control of *Campylobacter* in poultry and poultry meat is a major public health strategy in the prevention of human campylobacteriosis (EFSA, 2009, 2010, 2011a). Studies at slaughterhouse level have demonstrated that cross-contamination of the chicken carcasses may occur at scalding, evisceration and water-chilling stages which transfers the *Campylobacter* contamination to the retail level (Hue et al., 2010; Kudirkienė, Bunevičienė, Bruzdžiūtė, & Ingmér, 2011). In the United Kingdom, the joint government and industry target has been set to reduce *Campylobacter* contamination on whole chicken carcasses in slaughterhouses by 2015. More specifically, the target is based on *Campylobacter* counts, focusing on decreasing the proportion of birds in the most contaminated group i.e. >1000 CFU/g.

In 2011, the proportion of *Campylobacter* positive broiler meat samples varied widely between EU member states, from 3.2% (Austria) to 84.6% (Luxembourg), while the level of *Campylobacter* in broiler chicken flocks varied from 0% in Estonia to 92% in Slovenia (EFSA, 2013). An EU-wide baseline study showed the average *Campylobacter* prevalence for fresh broiler chicken carcasses to be 75.8% (EFSA, 2010). A worldwide literature survey has shown that about 58% of chickens are contaminated with *Campylobacter* (Suzuki & Yamamoto, 2009). Previous studies have shown differences between Estonia and the other Baltic countries for *Campylobacter* prevalence in fresh broiler chicken meat. In Estonia, *Campylobacter* prevalence in fresh chicken meat has been 15.8% from 2000 to 2002, and from 14% in 2003—2007 (Meremäe et al., 2010; Roasto, Praakle, Korkeala, Elias, & Hänninen, 2005). According to Kovalenko, Roasto, Liepins, Mäesaar, and Hörman (2013) 56.3% of broiler chicken carcasses were *Campylobacter*-positive in Latvia. A Lithuanian study by Bunevičienė, Kudirkienė, Ramonaitė, and Malakauskas (2010) reported higher than 40% *Campylobacter* contamination for fresh broiler chicken meat. Seasonal variation of *Campylobacter* occurrence in poultry has been previously found in Estonia and Latvia. A distinct seasonal peak in summer and in early autumn was found in Estonia (Meremäe et al., 2010) while Kovalenko et al. (2013) reported an increase in *Campylobacter* contamination in Latvia in early spring, while in late autumn contamination remained high during the summer months and decreased at the end of autumn. This is the first scientific notice reporting quantitative data on *Campylobacter* spp. contamination of fresh broiler chicken meat of Baltic origin sold at the retail level in Estonia.

The overall aim of this study was to determine the prevalence and seasonality of *Campylobacter* spp. contamination of poultry meat at the retail level in Estonia.

2. Materials and methods

Two surveys for *Campylobacters* in poultry and poultry meat were carried out in 2012 in Estonia. The results of these surveys were combined, taking into account the methodological and analytical characteristics of the survey protocols. In both surveys meat sampling was made at retail level in retail stores where most poultry meat is sold and purchased in Estonia for domestic consumption. Furthermore, sales proportions were taken into account while sampling. Mostly company-packaged fresh broiler chicken meat was collected at Estonian retail level. All twelve months were included, and for *Campylobacter* detection the same methodology was applied. However, the surveys differed in some aspects which are described below.

2.1. Sample collection

Of the two separate surveys, a total of 606 poultry meat samples at retail level and 380 broiler chicken caecal samples at slaughterhouse level were collected during the year 2012. Most of the collected poultry meat samples were from Estonia (44.1%), Lithuanian (43.2%) and Latvian (8.4%) origin, but some samples originated from Poland (1.7%), Germany (1.1%), Finland (0.9%), Belgium (0.3%), and Hungary (0.3%).

In contrast to the second survey, the first survey included not only broiler chicken meat samples (76.3%) but also turkey meat (18.7%), laying hen meat (4.7%) and duck meat (0.3%). The collected samples were from a range of poultry meat categories, including fresh meat (56.7%), carcasses (11.4%), minced meat (5%), meat preparations (24.9%) and heat-treated poultry meat products (2%). Furthermore, the second survey protocol included both *Campylobacter* detection and enumeration methods, and sampling also included broker chicken caecal sampling at slaughterhouse level for estimation of seasonality.

2.1.1. First survey

The first survey was organized by the Estonian Veterinary and Food Board, and included poultry meat sampling from retail outlets from throughout Estonia. In this survey 386 poultry meat samples were collected from different categories, such as fresh meat, carcasses, minced meat and meat preparations. Broiler chicken meat samples comprised 297 of the total of 386 samples. Meat samples were transported to the laboratory within sampling day in a portable cooler at a temperature of 4–6 °C; microbiological analyses began on the same day.

2.1.2. Second survey

The second survey was designed to estimate the prevalence and counts of *Campylobacter* spp. in high contamination-risk category products, such as fresh broiler chicken meat containing skin (drumsticks, wings and breast). Samples were collected from Estonian supermarket chain retail outlets. In the second survey only Estonian (53.6%), Lithuanian (37.3) and Latvian (9.1%) originating fresh broiler chicken meat samples were collected and analyzed. The proportion of other countries than Baltic fresh broiler chicken meat in Estonian retail is very small. Fresh broiler chicken meat sales proportions were taken into account while sampling at retail level. Estonian and Lithuanian products were available for purchase in all twelve months, while Latvian fresh broiler chicken products were available in Estonian retail outlets from September to December 2012. Only company–packaged fresh broiler chicken meat was sampled, in order to exclude the possibility of *Campylobacter* cross-contamination during storage. In total, 220 fresh poultry meat samples were collected within the 12 months of the second survey. To estimate *Campylobacter* colonization seasonality, 380 caecal samples were collected weekly from the slaughterhouse, which is owned by the company representing over 95% of all commercial broiler production in Estonia. This company owns six separate farms with 62 flocks in separate housing, with
approximately 22,000 birds per flock. Broiler chicken caecal material from randomly selected flocks (three pooled samples per farm) representing all six farms was collected from June to October, which was assumed to be the seasonal peak for Campylobacter contamination in Estonia. Caecal samples were taken from caecum blind sacs near the cloaca of the intestines of broiler chickens, and the material was directly transferred into tubes containing 10 ml of Bolton enrichment broth (Oxoid, Basingstoke, Hampshire, England). One loopful (10 μl) of caecal material was taken per bird, and the material from five birds was pooled into a single tube. Both caecal and meat samples were transported to the laboratory within sampling day in a portable cooler, and microbiological analyses began immediately on arrival of samples.

2.2. Isolation, identification and enumeration of Campylobacter spp.

The isolation of Campylobacter was carried out in two laboratories.

2.2.1. First survey

All analyses in the first survey were performed at the Estonian Veterinary and Food Laboratory. Campylobacter detection was carried out on 386 poultry meat samples according to the method described in ISO 10272-1:2006. The detection of Campylobacter was made primarily from the skin material, if available, and secondly from meat, depending on the sample type e.g. skinless poultry meat fillets. According to the detection method used, 10 g of skin or meat material was removed aseptically and placed into a sterile plastic bag. The plastic bag was then filled with 90 ml of sterile Bolton broth, and samples were processed for 1 min in a stomacher and then incubated, under microaerobic conditions, at 37 °C for 4 h—6 h, followed by 44 ± 4 h at 41.5 ± 0.5 °C. After enrichment, 10 μl of the enrichment broth was plated onto mCCDA agar (Oxoid; Basingstoke, Hampshire, England) and incubated for 24 h at 41.5 ± 0.5 °C and transported to the laboratory during sampling day. On arrival the tubes were immediately transferred into an incubator, and incubated at 41.5 ± 0.5 °C for 24 h in microaerobic conditions, following which Campylobacter detection and verification was carried out in accordance with ISO 10272-1:2006.

2.2.2. Second survey

The Laboratory of Food Hygiene of the Estonian University of Life Sciences analyzed 220 fresh broiler chicken meat samples and 380 caecal samples, collected from the second survey. The main difference compared to the first survey was that only broiler chicken meat skin material was used, and both Campylobacter detection and enumeration methods were applied. The detection of Campylobacter from fresh broiler chicken meat samples was carried out according to the ISO 10272-1:2006 method described above.

 Enumeration was carried out according to the method described in ISO 10272-2:2006. In brief, 0.1 ml of 10⁻¹ and 10⁻² broiler chicken meat skin dilutions were streaked onto modified CCDA agar and incubated for 44—48 h at 41.5 ± 0.5 °C. Randomly selected five presumptive Campylobacter colonies were further subcultured on Columbia blood agar and later identified by microscopic examination, gram staining, and biochemical tests, as described in the ISO method.

Additionally, Bolton enrichment broth tubes with pooled caecal material were held at −82 °C in glycerol broth (20% [vol/vol] glycerol in 1% [wt/vol] protease peptone).

2.3. Campylobacter species identification

Conventional multiplex PCR assay was used for identification and differentiation of C. jejuni, C. coli, Campylobacter lari, Campylobacter upsaliensis, and Campylobacter fetus subsp. fetus, as described by Wang et al. (2002).

2.4. Statistical analysis

All individual results were recorded using MS Excel 2010 software (Microsoft Corporation, Redmond, Wash.), and statistical analysis was performed with the Statistical Package R in order to determine if there were statistically significant differences at 95% and 99% confidence levels in the prevalence and counts of Campylobacter in the poultry products of different origin using the Kruskal–Wallis rank sum test and Chi-square test. Additionally, seasonal variation in Campylobacter contamination was analyzed in order to elucidate differences in prevalence’s between different sampling months.

3. Results and discussion

3.1. First survey

Among 386 poultry meat samples, the mean proportion of Campylobacter positive samples was 12.7% (Table 1). The proportion of Campylobacter contamination was 25.8% for Latvian products,

<table>
<thead>
<tr>
<th>Country of the origin</th>
<th>No. of positive samples/No. of all samples (positive %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fresh meat</td>
</tr>
<tr>
<td>Estonia</td>
<td>10/56 (17.9)</td>
</tr>
<tr>
<td>Lithuania</td>
<td>14/125 (11.2)</td>
</tr>
<tr>
<td>Latvia</td>
<td>5/22 (22.7)</td>
</tr>
<tr>
<td>Otherb</td>
<td>0/16 (0.0)</td>
</tr>
<tr>
<td>In total</td>
<td>29/219 (13.2)</td>
</tr>
</tbody>
</table>

NS, No samples available.

a Survey conducted by the Estonian Veterinary and Food Board.

b Poland, Germany, Finland, Belgium, Hungary.
14.8% for Estonian products, 10.6% for Lithuanian products and 0% for poultry products from countries other than the Baltic countries. Among raw poultry products fresh broiler chicken meat of Baltic origin is mostly sold in the Estonian retail market. The contamination of fresh broiler chicken meat of Estonian, Lithuanian and Latvian origin was 17.0%, 9.1% and 22.7% respectively. Within the tested meat categories, highest Campylobacter contamination was found in minced meat (23.8%) followed by fresh meat (13.2%), whole carcasses (11.4%) and meat preparations (10.4%). Sampling of the heat-treated poultry meat products was not included in the first survey sampling plan. These samples (n = 6) were taken by official veterinarian unintentionally, and as it is expected all were Campylobacter negative.

3.2. Second survey

Among 220 fresh broiler chicken meat samples (drumsticks, wings and breast), the mean proportion of Campylobacter positive samples was 35% (Table 2). Among Estonian, Lithuanian and Latvian-origin broiler chicken meat samples the proportions of Campylobacter positive products were 20.3%, 50.0% and 60.0%, respectively. The number of analyzed Latvian products in this study was small because Latvian-origin fresh broiler chicken meat products were available for purchase at the Estonian retail level only from September to December in 2012. Nevertheless, similarly to these results, almost 60% of Campylobacter prevalence in fresh broiler chicken meat was reported in a recent Latvian study (Kovalenko et al., 2013).

According to EFSA scientific opinion (2011a) a public health risk reduction of >50% or >90% could be achieved if all broth samples were to comply with a microbiological criterion of a critical limit of 1000 or 500 CFU/g of neck and breast skin, respectively. The results of the Campylobacter enumeration on fresh broiler chicken meat in this study were categorized as follows: < 100 CFU/g; 100–499 CFU/g; 500–1000 CFU/g and >1000 CFU/g. An EU baseline survey reported that Campylobacter counts on broiler carcasses of Estonian origin were <10 CFU/g in 98% of positive cases (EFSA, 2010). In the current study higher Campylobacter contamination levels for Estonian broiler chicken products were found (Table 3). Enumeration results, in the case of positive results from enumeration analyses, showed that the overall arithmetic Campylobacter CFU mean was 3.2 log10 CFU/g of product (Table 2) with the highest mean contamination loads in Latvian-origin products and the lowest in those from Estonia, respectively 3.4 log10 CFU/g and 2.8 log10 CFU/g. The mean contamination load for Lithuanian-origin broiler chicken products available at Estonian retail was 3.2 log10 CFU/g. A previous Lithuanian study (Bunevičienė et al., 2010) reported lower Campylobacter counts (mean 2.0 log10 CFU/g) in broiler chicken meat. Among Estonian, Lithuanian and Latvian-origin products, with positive enumeration results, a contamination level of above 1000 Campylobacter CFU/g was found in 1.7%, 14.6% and 35.0% of samples respectively.

3.3. Both surveys

Fresh broiler chicken meat contamination in the first and second surveys was 13.5% and 35%. This difference can be explained by differences in the sampling methods. In the second survey only skin material from drumsticks, wings and breast portions was analyzed for Campylobacter. The first survey also included fillets and other fresh broiler meat products without skin material. It is well-known that Campylobacter spp. can colonize the intestinal tract of broiler chickens at high levels, and during a poorly executed evisceration process at slaughter the caecal material can be transferred onto carcasses (Allen et al., 2007; Reich, Atanassova, Haunhorst, & Klein, 2008). A Finnish study by Katzav, Isohanni, Lund, Hakkinen, and Lyhns (2008) showed the occurrence of Campylobacter in chicken slices and barbecue sticks to be 9.4%, in chicken breast fillets 4.7% and in chicken products with skin and bone 30.4%. The results of the present study showed that the percentage of Campylobacter-positive fresh broiler meat samples was higher when skin material was included. Within company-packaged fresh broiler chicken meat sold at Estonian retail level the majority (~70%) is sold as drumsticks, wings and breast portion where the skin material is included.

According to an EU-wide baseline survey (EFSA, 2011b) the Campylobacter prevalence for broiler chicken batches in Estonia was only 2.0%, which was the lowest among the EU-countries. According to the first and second surveys of the present study, Campylobacter prevalence in fresh broiler chicken meat of Estonian origin was 17.0% and 20.3%. Differences compared to the prevalence found in the EU-baseline study are also probably related to the different sampling methods. For the baseline study broiler chicken carcasses were collected and neck skin samples taken at the laboratory for Campylobacter prevalence, instead of company-packaged broiler fresh meat samples (drumsticks, wings, breast portions) that were used in the surveys presented here. It is not well-known how representative the neck and breast-skin are for estimating whole-carcass regions, and which sampling schemes, e.g. number of samples or time interval between sampling, are most effective. Jørgensen et al., (2002) reported that the likelihood of detecting Campylobacter spp. in a raw chicken appeared not to be significantly influenced by sample type, but examination of samples containing carcass rinse fluid and neck-skin detected a higher Campylobacter count than examination of the neck-skin sample alone. Because of non-similarities in study design, the Jørgensen et al., (2002) findings cannot explain the differences found in the currently described surveys. Nevertheless, the proportion of Campylobacter positive Estonian origin broiler chicken carcasses in the first survey was 5% (Table 1), which is more comparable to the EU base-line study Estonian results where the Campylobacter

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**Table 2**

<table>
<thead>
<tr>
<th>Country of origin</th>
<th>No. of positive samples/No. of all samples (positive %)</th>
<th>Enumeration results of positive samples, log10 CFU/g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>Estonia</td>
<td>24/118 (20.3)</td>
<td>2.8</td>
</tr>
<tr>
<td>Lithuania</td>
<td>41/82 (50.0)</td>
<td>3.2</td>
</tr>
<tr>
<td>Latvia</td>
<td>12/20 (60.0)</td>
<td>3.4</td>
</tr>
<tr>
<td>Total</td>
<td>77/220 (35.0)</td>
<td>3.2</td>
</tr>
</tbody>
</table>

* Survey conducted by the Estonian University of Life Sciences.
* * Samples with positive detection and positive enumeration result, the threshold of 100 CFU/g.
prevalence was 2%. It is essential to note that most of the broiler chicken fresh meat in Estonia is sold as packaged drumsticks, wings and other portions instead of whole carcasses.

**Campylobacter** studies in other Baltic countries have shown high Campylobacter occurrence in Latvia, where the mean proportion of Campylobacter positive broiler chicken carcasses at Latvian retail level was 59.2% in 2010, as reported by Kovalenko et al. (2013). A Lithuanian study by Bunevičienė et al. (2010) showed that fresh broiler chicken meat (drumsticks and wings) at the retail level were contaminated at up to 46.5%, and broiler chicken carcasses at slaughterhouse level at up to 45.8%. These studies report similar contamination proportions to the second survey reported here, where Campylobacter contamination in Latvian and Lithuanian fresh broiler chicken meat was 60% and 50%, respectively.

The prevalence’s of Campylobacter spp. on fresh broiler chicken meat samples of Estonian and Lithuanian origin (data available for all 12 months, both surveys combined) during the year 2012 are shown in Fig. 1. It is possible to deduce that Campylobacter spp. contamination increased in early spring, remained high during the summer months, and decreased at the end of autumn. June 2012 was atypically rainy and cold in the Baltic countries, which may be a possible reason for the sudden decrease in Campylobacter positive broiler chicken meat samples shown in Fig. 1. The seasonal variation of Campylobacter contamination was also studied at slaughterhouse level, where the caecal samples were taken at assumed seasonal peaks, from June to October. Among the Estonian broiler chicken farms and flocks at the slaughterhouse level studied, the overall prevalence’s of Campylobacter in caecal material monthly from June to October were 0%, 39%, 92%, 45% and 0%, respectively. The prevalence’s of Campylobacter in Estonian fresh broiler chicken meat (Fig. 1), in the same months, were 0%, 16.7%, 75.0%, 41.7% and 22.2%. Generally, the higher the Campylobacter contamination was at farm level the higher it was in broiler chicken meat samples at retail level.

There was seasonal variation in the proportions of Campylobacter positive samples with a seasonal peak in the warm summer months of July, August and September (p < 0.001). A distinct seasonality in broiler chicken Campylobacter contamination and in human campylobacteriosis cases have been shown by previous European studies (Horrocks, Anderson, Nisbet, & Ricke, 2009; Rautelin & Hänninen, 2000) and in New Zealand (Brieseman, 1990). Reports on human campylobacteriosis cases in Estonia have shown that most Campylobacter human infections occurred from June to September (Meremäe et al., 2010), the season when the highest Campylobacter prevalence and counts of the poultry products at Estonian retail level were found in the current study. Campylobacter counts (second survey), between Estonian, Lithuanian and Latvian fresh broiler chicken products (n = 220), were compared. Significant differences for Campylobacter prevalence and counts between Estonian and Lithuanian (p < 0.001) and between Estonian and Latvian (p < 0.001) fresh poultry products were found. No statistical difference was found for Campylobacter contamination, both counts and prevalence, between Latvian and Lithuanian-origin products. Estonian fresh poultry meat products had significantly (p < 0.001) lower Campylobacter prevalence and counts compared to Lithuanian and Latvian poultry products sold at Estonian retail. The reasons for the higher Campylobacter contamination for Latvian and Lithuanian fresh poultry meat were not part of the study, but are probably related to differences in production and management systems at poultry farm, slaughterhouse and at meat industry levels. Data presented in Fig. 1 show a high proportion of Campylobacter positive Lithuanian-origin broiler chicken meat samples in December. One possible explanation for this could be the fact that the lowest number of samples was taken in December, and so the data was least reliable for this month, but the same number of samples were collected both for Estonian and Lithuanian products. Nevertheless, the proportion of Campylobacter positive products was 16.7% for Estonian and 60% for Lithuanian-origin fresh broiler chicken meat products.

Campylobacter species distribution among poultry products originating from different countries may be different. Therefore, a PCR assay for Campylobacter species identification, described by Wang et al. (2002), was performed which resulted in estimations of 89% C. jejuni, 8% C. coli and 3% Campylobacter spp. isolates, with no essential differences between countries of origin.

4. Conclusion

High numbers of Campylobacter on fresh broiler chicken meat of Latvian and Lithuanian origin were found in the Estonian retail market.

Campylobacter prevalence in fresh broiler chicken meat of Estonian origin was lower compared to most EU-countries, but higher than previously reported by the EFSA. The seasonal peak for Campylobacter contamination of poultry meat was in the summer.

To achieve EU targets in public health risk reduction in Estonia, appropriate Campylobacter control measures should be applied at all broiler chicken meat production stages, with a special emphasis on the warm summer months.

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