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High occurrence of *Campylobacter* spp. in Latvian broiler chicken production

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ABSTRACT

Campylobacteriosis in humans is caused by thermotolerant *Campylobacter* spp., most commonly by *C. jejuni* and *C. coli*. However, no official data for human campylobacteriosis in Latvia is available or cases are heavily under-reported. In accordance with Commission Decision 2007/516/EC the *Campylobacter* spp. baseline study was performed in 2008 in Latvia but there was no continuous monitoring for the *Campylobacter* at broiler chicken production level in 2009. The aim of the present study was to determine the occurrence of *Campylobacter* spp. in broiler chicken production at slaughterhouse and retail level in Latvia. Poultry samples originated from the two biggest Latvian broiler slaughterhouses. Altogether, 240 fresh broiler chicken neck skins, 2400 intact broiler chicken intestines and 240 fresh broiler chicken carcasses were collected during the year 2010. A total of 92.5% of the pooled intestine samples; 60.8% of the neck skin samples and 56.3% of carcasses were positive for *Campylobacter* spp. There was seasonal variation in proportions of *Campylobacter* positive samples with seasonal peak on summer months.

1. Introduction

Campylobacteriosis in humans is caused by thermotolerant *Campylobacter* spp. and from all *Campylobacter* species *C. jejuni* and *C. coli* are the most commonly reported bacterial causes of human intestinal infections in European Union (EU). In average 48.6 confirmed campylobacteriosis cases per 100,000 EU inhabitants were reported in 2010 (EFSA, 2012). In most cases *Campylobacter* spp. cause gastroenteritis in humans but in a few incidences *Campylobacter* infection may also cause post-infection complications like Miller-Fisher and Guillain–Barré syndrome that can lead to serious health issues and even death (Fica et al., 2011; Kuwabara, 2011).

C. coli and *C. jejuni* are slender, spirally curved, gram-negative rods with a characteristic corkscrew-like darting motility. Compared to other food-borne bacterial pathogens, *Campylobacter* are more fragile and require microaerobic conditions for multiplication (Park, 2002).

The most important source of these bacteria is related with poultry meat; therefore the control of *Campylobacter* in poultry meat is a major public health strategy for the prevention of human campylobacteriosis (Friedman et al., 2004). In 2010, the proportions of *Campylobacter*-positive broiler meat samples varied widely between European Union member states (EU), from 3.1% to 90%, while the level of *Campylobacter* in broiler chicken flocks varied from 0% in Estonia to 92.9% in Slovenia. In accordance with EU-wide baseline study the average *Campylobacter* prevalence for fresh broiler chicken carcasses was 75.8% (EFSA, 2011 and 2012).

Poultry is exposed to the *Campylobacters* usually first at the farm level and the exposure is directly related to the insufficient biosecurity measures in and around the poultry farm (Ellis-Iversen et al., 2009; Newell & Fearnley, 2003). In a flock with 20,000 broilers the prevalence of *Campylobacter* can increase from 5% to 95% within the 6 first days after initial *Campylobacter* introduction (Van Gerwe et al., 2005). At the slaughterhouse level the cross-contamination of the chicken carcasses has been observed at scalding, evisceration and water chilling stages following by the transmission of the *Campylobacter* contamination to the retail level (Hue et al., 2010; Jacobs-Reitsma, 2000). Studies done in Estonia and Lithuania showed different seasonal variations of *Campylobacter* occurrence, the highest occurrence being in winter and spring months in Lithuania and in summer and early autumn in Estonia (Meremäe et al., 2010; Pieskus, Butrimaite-Ambrazeviciene, & Kazeniauskas, 2008).

The aim of the present study was to determine the occurrence of *Campylobacter* spp. in broiler chicken production at slaughterhouse and retail level in Latvia in 2010.

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2. Materials and methods

2.1. Sampling

A total of 240 fresh broiler chicken neck skins, 2400 whole broiler chicken intact intestines and 240 fresh broiler chicken carcasses were collected during the year 2010. All the samples were collected monthly and in each month 10 broiler chicken neck skin samples and 100 intact intestines at a random basis from each of two investigated broiler chicken meat company slaughterhouse were collected. The chickens sampled in the study were from companies that produce more than 75% of all commercial broilers in Latvia.

All intact broiler chicken intestines were taken at the time of evisceration. The neck skin samples were taken and placed separately in sterile plastic bags while ten intact intestines were placed in a single sterile plastic bag for transport. Intestine samples and neck skin samples were collected at the same day and were a part of the same slaughter batch. Additionally, each month 10 fresh broiler chicken carcasses from the production of the same broiler meat producers were collected at retail level of Latvia. Carcass samples were mostly collected at the same day as the sampling in slaughterhouses was performed, but they did not represent the same slaughter batch as the intestine samples and neck skin samples. Broiler chicken carcasses from slaughterhouse 'A' production were sold in tight, sealed plastic bags opposite to the slaughterhouse 'B' where broiler chicken carcasses were sold in lose, unsealed plastic bags. All the samples were transported to the laboratory after being placed in a portable cooler at a temperature 4–6 °C and microbiological analyses were carried out immediately after arrival to the laboratory in accordance with good laboratory practices.

2.2. Isolation and identification of Campylobacter spp.

Altogether 960 *Campylobacter* analyses were performed; respectively 480 from caecal material, 240 neck skin and 240 from broiler chicken carcasses.

The isolation of *Campylobacter* was carried out in the Food Hygiene laboratory of the Institute of Food and Environmental Hygiene, Latvian University of Agriculture (Jelgava, Latvia) using the following procedures. Sample transport after sampling from slaughterhouse to research laboratory lasted from 2 to maximum 4 h. Immediately after transport 10 g of neck skin material and in the case of broiler chicken carcass 10 g of chicken back skin was aseptically taken and placed into sterile plastic bag for enrichment. Plastic bag were then filled with 90 mL of sterile Bolton broth (Oxoid; Basingstoke, Hampshire, UK), and the samples were processed for one minute in a stomacher and then incubated under microaerobic conditions at 37 °C for 46 h, followed by 41.5 °C for 44 ± 4 h. After enrichment, 10 μL of the enrichment broth was plated on mCCDA agar (Oxoid; Basingstoke, Hampshire, England) and incubated for 48 h at 42 ± 0.5 °C under microaerobic conditions. Typical *Campylobacter* colonies on mCCDA plates were streaked on Columbia blood agar (Oxoid) plates, which were incubated for 24 h at 41.5 °C in microaerobic conditions using anaerobic jars and CampyGen™ reagents (Oxoid). After transportation to the laboratory randomly one intestine from 10 broiler chicken intact intestines was dissected to analyse separately from the pooled chicken intestine samples. From 10 intestines caeca were dissected and caecal material from 10 caeca was pooled together for one composite sample and 1 g of the content was further analysed. All the analyses and confirmation tests were performed in accordance with instructions of the detection method described by ISO 10272-1:2006. The bacteria isolated from broiler chicken material that showed typical growth on mCCDA, were gram negative, had corkscrew-like darting motility, were oxidase positive and did not show growth at 41.5 °C in aerobic conditions and growth at 25 °C in microaerobic conditions, were considered as *Campylobacter* spp.

2.3. 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% level in the prevalence of the *Campylobacter* positive samples between the two slaughterhouses and between the production stage at these slaughterhouses by using the Chi-square test.

3. Results and discussion

Present study resulted in detection of high *Campylobacter* colonization for caecal samples and high contamination of neck skin samples in two biggest Latvian broiler chicken slaughterhouses. The proportions of *Campylobacter* spp. positive broiler chicken caecal and neck skin samples at slaughterhouse level and broiler chicken carcass samples at retail level in 2010 is shown in Table 1.

The average proportion of *Campylobacter* positive broiler chicken neck skin samples from slaughterhouse 'A' was significantly ($p < 0.05$) higher than in chicken neck skin samples from slaughterhouse 'B'. According to the data shown in Table 1 we can conclude that there were no statistically significant differences in the level of the initial prevalence of campylobacters in the separate or pooled fecal samples between the slaughterhouses. There were statistically significantly less positive neck skin and carcass samples (and in all samples together) in the slaughterhouse B than in slaughterhouse A. Although, the overall trend was that there were less *Campylobacter* positive samples in the neck skin samples than in the fecal samples and also less in the carcass samples than in both fecal and neck skin samples. Only in the slaughterhouse B this observation is statistically significant. The higher level of *Campylobacter* positive caecum samples compared with the neck skin samples was unexpected but the possible reasons are not

Table 1
The proportion of *Campylobacter* spp. positive broiler chicken samples at Latvian slaughterhouse and retail level in 2010.

Slaughterhouse	*Campylobacter* spp. positive samples % (No. positive/total No.)				
	Separete fecal samples	Pooled fecal samples	Neck skin samples	Carcass samples	All samples
A	75.0 (90/120)	94.2 (113/120)	69.2 (83/120)	65.8[a] (79/120)	76.0 (365/480)
B	62.5 (75/120)	90.8 (109/120)	52.5 (63/120)	46.7[b] (56/120)	63.1 (303/480)
All	68.8 (165/240)	92.5 (222/240)	60.8 (146/240)	56.3 (135/240)	69.6 (668/960)
p-Value for the difference A–B	$p > 0.05$	$p > 0.05$	$p < 0.05$	$p < 0.01$	$p < 0.01$

[a] $p > 0.05$ for the difference between the separate fecal samples and the carcass samples.
[b] $p < 0.05$ for the difference between the separate fecal samples and the carcass samples.