THE EFFECT OF AIR TEMPERATURE ON THE OCCURRENCE OF THERMOPHILIC *CAMPYLOBACTER* SPP. IN LATVIAN BROILER CHICKEN PRODUCTION ON DAY OF SAMPLING

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ABSTRACT
Campylobacteriosis in humans is caused by thermophilic *Campylobacter* spp., most commonly by *C. jejuni*. The aim of the present study was to determine the effect of the average air temperature of sampling day on the occurrence of *Campylobacter* spp. in broiler chicken production at slaughterhouses and retail level in Latvia. Poultry samples originated from the two biggest Latvian broiler slaughterhouses. In all, 240 fresh broiler chicken neck skins, 240 intact broiler chicken intestines and 240 fresh broiler chicken carcasses were collected during the year 2010. In total, 68.8% of the intestine samples, 60.8% of the neck skin samples, and 56.3% of carcasses were positive for *Campylobacter* spp. with a high correlation ($r \geq 0.65$; $p < 0.05$) of air temperature on day of sampling.

KEY WORDS: *Campylobacter*, temperature, broiler chicken, slaughterhouse, retail.

INTRODUCTION
Campylobacteriosis in humans is caused by thermophilic *Campylobacter* spp., and from all *Campylobacter* species *C. jejuni* is the most commonly reported bacterial cause of human intestinal infections in European Union (EU). On average, 48.6 confirmed campylobacteriosis cases per 100,000 EU inhabitants were reported in 2010 (EFSA, 2012).

Thermophilic *Campylobacter* are thin, 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 to 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).

Poultry is usually exposed to the *Campylobacter* first at the farm level, and it is directly related to the insufficient biosecurity measures on and around the poultry farm (Newell & Fearnley, 2003; Ellis-Iversen *et al.*, 2009). 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 (Pieskus, Butrimaite-Ambrazeviciene & Kazeniauskas, 2008; Meremäe et al., 2010).

The aim of the present study was to determine the effect of the air temperature of sampling day on the occurrence of *Campylobacter* spp. in broiler chicken production at slaughterhouse and retail level in Latvia.

MATERIAL AND METHODS

In total, 240 fresh broiler chicken neck skins, 240 whole broiler chicken intact intestines and 240 fresh broiler chicken carcasses were collected during the year 2010. All the samples were collected monthly at 5pm ±1h. Every month 10 broiler chicken neck skin samples and 10 intact intestines were collected at a random basis from each of two investigated broiler chicken meat company slaughterhouses. These companies produce more than 75% of all commercial broilers in Latvia and are situated 35km and 40 km in a straight line from city Jelgava (Latvia), where broiler chicken carcass samples were collected. The air temperature of sampling day at 5 pm in Jelgava was recorded according to the data provided by VSIA „Latvijas Vides, ģeoloģijas un meteoroloģijas centrs."

Ten intact broiler chicken intestines were taken at the time of evisceration and placed in a single sterile plastic bag for transportation. The neck skin samples were taken and placed separately in sterile plastic bags. Intestine samples and neck skin samples were collected at the same day and were part of the same slaughter batch. In addition, every month 10 fresh broiler chicken carcasses from the production of the same broiler meat producers were collected at two supermarkets in Jelgava. Carcass samples were collected on 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. 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 a good laboratory practice.

Isolation and identification of *Campylobacter* spp.

In all, 720 *Campylobacter* analyses were performed: 240 from caecal material, 240 neck skin and 240 from broiler chicken carcasses.

The isolation of *Campylobacter* was carried out 2 to 4 hours after sampling in the Food Hygiene laboratory of the Institute of Food and Environmental Hygiene, Latvian University of Agriculture (Jelgava, Latvia) by using the instructions of the detection method described by ISO 10272-1:2006. Ten grams of the neck skin material and 10 grams of the broiler chicken carcass back skin was aseptically taken and placed into sterile plastic bags for enrichment. Then plastic bags were filled with 90 ml of sterile Bolton broth (Oxoid; Basingstoke, Hampshire, UK), and the samples were processed for one minute in a stomacher, and incubated under microaerobic conditions at 37 °C from 4 h to 6 h, followed by 44 ± 4 h at temperature 41.5 °C. 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 ºC 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^{TM} reagents (Oxoid). Caecal contents was analyzed by direct plating of 10 µg of the caecal contents on mCCDA agar as described in 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 thermophilic *Campylobacter* spp.

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 correlation and statistical significance at 95% and 99% level between the prevalence of the *Campylobacter* positive samples and sampling day air temperature by using Pearson correlation test.

RESULTS AND DISCUSSION

In total, 68.8% of the intestine samples, 60.8% of the neck skin samples and 56.3% of carcasses were positive for *Campylobacter* spp. The highest occurrence (83.3%) of *Campylobacter* positive samples was observed in August and June. Additionally, the effect of air temperature on occurrence of *Campylobacter* spp. in broiler chicken fecal and meat samples was established. Statistical analyses showed a high correlation ($r \geq 0.65$) between the air temperature on sampling day at 5 pm and occurrence of *Campylobacter* spp. in broiler chicken samples. The highest correlation was observed in fecal samples ($r=0.75$; $p<0.005$). *Campylobacter* occurrence in the neck skin samples showed lower correlation ($r=0.66$; $p<0.01$). The lowest correlation was stated in carcass samples ($r=0.65$; $p<0.05$). The current study found that thermophilic *Campylobacter* spp. could be isolated when the air temperature of the sampling day was 10 to 18°C (Figure 1.).

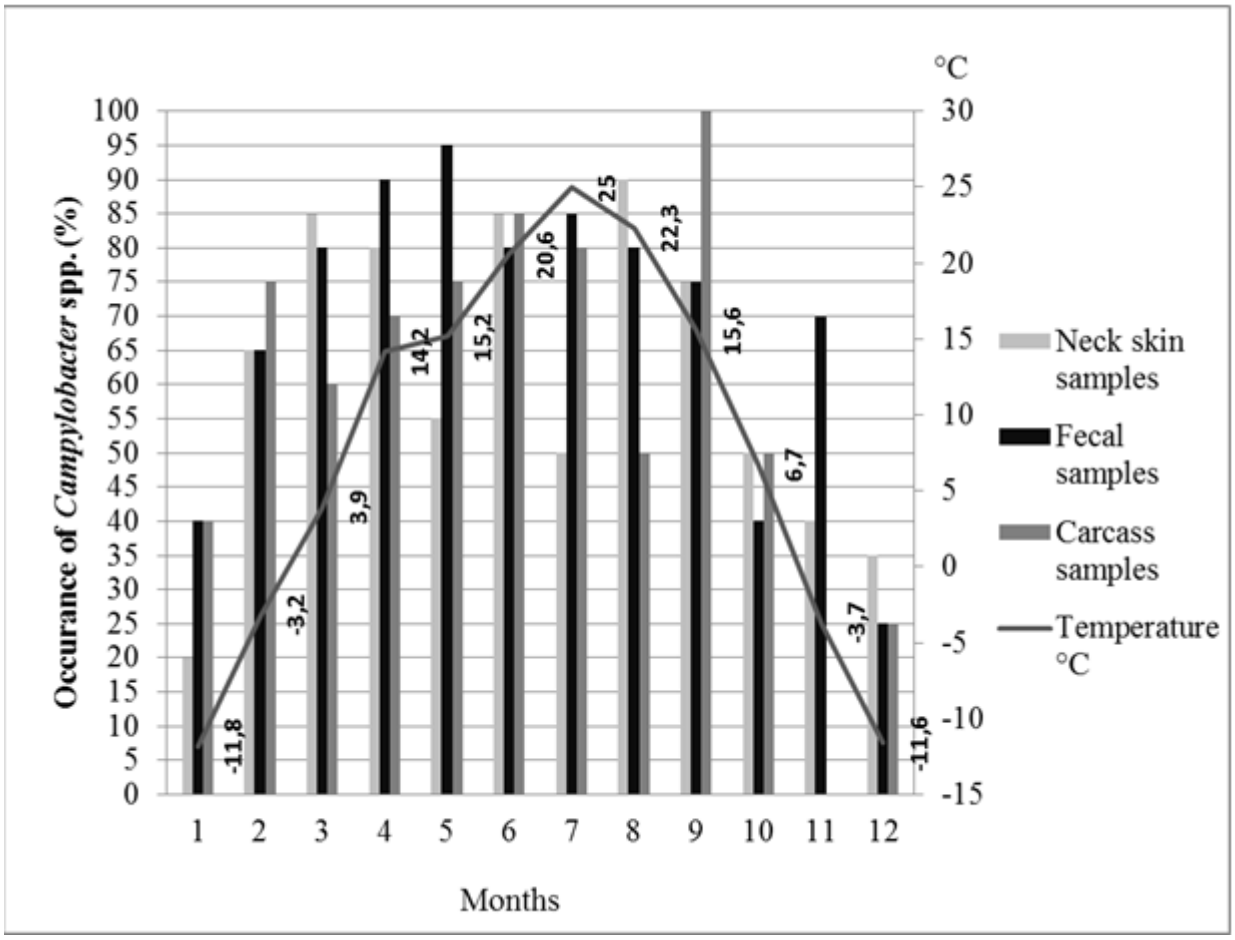

Figure 1. **Occurrence of *Campylobacter* positive samples and air temperature of sampling day**

Both for fecal samples and neck skin samples, the highest occurrence of *Campylobacter* positive samples was observed in spring, summer and autumn months, with significantly ($p>0.05$) lower incidence in winter when the air temperature was the lowest (Figure 1). In temperate climate zones, the highest occurrence of *Campylobacter* contamination is usually observed during the warm months of the year, when the highest number of campylobacteriosis cases in humans is registered (Friedman et al., 2000; EFSA, 2012; EFSA, 2011; EFSA, 2009).

Slight seasonal differences can be noticed when comparing the *Campylobacter* occurrence data in broiler carcasses in Latvia with the occurrence of *Campylobacter* on carcasses and meat samples in Estonia. In Estonia, the highest proportion of positive samples was determined in June, July and August as well as in the Nordic countries such as Sweden, Finland, Denmark and Norway (Roasto et al., 2005; Rautelin and Hänninen, 2000; Wingstrand et al., 2006). Meanwhile, *Campylobacter* was found all year round without significant seasonal variations from the meat that was imported into Estonia and was commercially available in the market in Tartu (Roasto et al., 2005). The seasonal variation of the *Campylobacter* occurrence in Latvia as well as in other temperate climate zone countries can be explained by the air temperature differences of the seasons and the specific characteristics of *Campylobacter* to remain viable in products and poultry housings. Viability of *Campylobacter* directly depends on the air temperature, as Norwegian and Icelandic studies had found that air temperature higher than 6 °C in Norway, or 4 °C in Iceland, affected significantly the increase of the occurrence of *Campylobacter* on broiler chicken farms and in the slaughterhouse (Jonsson et al., 2012, Guerin et al., 2008). Correlation between the air temperature and the occurrence of *Campylobacter* spp. on carcass samples was weaker and the exact reasons are not known. Probably it can be related to different slaughter hygiene aspects.

CONCLUSION

The present study detected high correlation ($r \geq 0.65$) between the air temperature on sampling day and the occurrence of *Campylobacter* spp. in broiler chicken samples at slaughterhouse and retail level in Latvia.

ACKNOWLEDGEMENTS

Academic study and publication is financed by:
Project ‘Support for doctoral studies in LLU’ /2009/0180/1DP/1.1.2.1.2/09/IPIA/VIAA/017/ agreement No.044-08/EF2.D4.01;
Estonian Scientific Council (Eesti Teadusagentuur) Grant No. 9315.

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