OCCURRENCE AND NUMBERS OF *CAMPYLOBACTER* SPP. ON WINGS AND DRUMSTICKS OF BROILER CHICKENS AT THE RETAIL LEVEL IN LITHUANIA

Jurgita Bunevičienė[1*], Eglė Kudirkienė[1], Sigita Ramonaitė[2], Mindaugas Malakauskas[1]
[1]*Department of Food Safety and Animal Hygiene, Lithuanian Veterinary Academy*
Tilžės str. 18, Kaunas LT-47181, Lithuania, Tel. +370 37 363208; E-mail: buneviciene@lva.lt
[2]*Department of Infectious Diseases; Lithuanian Veterinary Academy*
Tilžės str. 18, Kaunas LT-47181, Lithuania

Summary. The present study was designed to investigate the occurrence and numbers of *Campylobacter* spp. on broiler chicken wings and drumsticks at the retails level of the three main poultry meat producers in Lithuania. Samples of chicken wings and drumsticks were collected of each poultry meat producer by visiting randomly selected retail shops once a week from March till October, in 2009. During each visit to a shop, samples of wings and drumsticks were bought without giving a warning. A total of 87 chicken wings and 87 drumsticks samples were collected and tested for *Campylobacter* spp. Thermophilic *Campylobacter* spp. were isolated by both direct inoculation on mCCDA selective medium and by selective enrichment in Bolton enrichment broth. Multiplex- PCR method was used for detection and identification of thermophilic *Campylobacter* species.

Our findings showed that overall 46.55% (81 out of 174) of the collected samples were contaminated with campylobacters. Twenty nine samples out of 81 were confirmed as positive only after enrichment procedure. *C. jejuni* has been found in 69.12% of the tested samples and *C. coli* in 13.23%, and both species together in 17.65%, respectively. The mean number of *Campylobacter* bacteria detected on wings at the retail was 1.99 log10 CFU/ml and on drumsticks 2.11 log10 CFU/ml.

This study shows high occurrence of broiler meat contamination with *Campylobacter* spp. at a retail level in Lithuania. Therefore the risk for consumers should be evaluated and an improvement of control measures at poultry production and retail level should be considered to reduce the risk for consumer's infection with *Campylobacter* spp.

Keywords: *Campylobacter* spp., chicken, retail, occurrence, contamination.

LIETUVOS PARDUOTUVĖSE PARDUODAMŲ BROILERIŲ SPARNELIŲ IR BLAUZDELIŲ UŽKRĖSTUMAS KAMPILOBAKTERIJOMIS

Jurgita Bunevičienė[1*], Eglė Kudirkienė[1], Sigita Ramonaitė[2], Mindaugas Malakauskas[1]
[1]*Maisto saugos ir gyvūnų higienos katedra, Lietuvos veterinarijos akademija*
Tilžės g. 18, LT-47181 Kaunas, Lietuva; tel. (8~37) 36 32 08; el. paštas: buneviciene@lva.lt
[2]*Užkrečiamųjų ligų katedra, Lietuvos veterinarijos akademija, Tilžės g. 18, LT-47181 Kaunas, Lietuva*

Santrauka. Mūsų tyrimo tikslas – įvertinti Lietuvoje gaminamų ir parduodamų paukštienos produktų užkrėstumą termofilinėmis kampilobakterijomis. Pasirinkti trys paukštienos gamintojai Lietuvoje. Jų gaminiai – broilerių sparneliai bei blauzdelės, gamintojų neįspėjus, buvo pirkti parduotuvėse vieną kartą per savaitę, 2009 m. kovo–rugsėjo mėnesiais. Iš viso ištirta 174 paukštienos produktai, iš jų 87 vnt. broilerių sparnelių ir 87 vnt. blauzdelių. Kiekvieno mėginio termofilinės kampilobakterijos aptiktos jas tiesiogiai sėjant ant „mCCDA" agaro ir pagausinant „Bolton" sultiniu. Kampilobakterijų padermės iki rūšies identifikuotos dauginės polimerazės grandininės reakcijos metodu.

Nustatyta, kad 46,55 proc. visų tirtų paukštienos produktų buvo užkrėsti termofilinėmis kampilobakterijomis. Identifikuojant išskirtas kampilobakterijų padermes, *C. jejuni* nustatyta 69,12 proc., *C. coli* – 13,23 proc., o abi padermės kartu – 17,65 proc. užkrėstų mėginių. Kiekybinio paukštienos produktų tyrimo rezultatai parodė, kad vidutinis *Campylobacter* spp. skaičius, tiriant broilerių sparnelius ir blauzdeles, buvo atitinkamai 1,99 log10 ksv/ml ir 2,11 log10 ksv/ml.

Šio tyrimo metu nustatytas sąlyginai didelis paukštienos produktų užkrėstumas termofilinėmis kampilobakterijomis rodo, kad parduodami žali broilerių produktai (sparneliai ir blauzdelės) gali kelti grėsmę žmonėms užsikrėsti kampilobakterioze, jei nebus laikomasi terminio apdorojimo reiklavimų ir higienos taisyklių. Norint sumažinti vartotojų riziką užsikrėsti kampilobakterioze per broilerių produktus, būtina sumažinti kampilobakterijų paplitimą broilerių pulkuose ir taip skatinti neužkrėstos paukštienos gamybą.

Raktažodžiai: *Campylobacter* spp., paukštiena, prekyba, užkrėstumas.

Introduction. *Campylobacter* spp. is the leading cause of bacterial food borne diarrheal diseases throughout the world (Skovgaard, 2007). In 2008, a total of 190,566 confirmed cases of campylobacteriosis were reported from 25 European countries, corresponding to an EU incidence of 40.7 campylobacteriosis cases per 100 000 population. In Lithuania, campylobacteriosis is one of the three most prevalent food borne zoonoses in humans with the incidence of 22.6 cases per 100 000 population although salmonellosis and yersiniosis have been detected

more frequently (EFSA 2010). Human campylobacter infections usually associated to the consumption of undercooked poultry meat or handling of contaminated poultry products (Altekruse et al., 1999, Friedman et al. 2004, Wingstrand et al. 2006). *Campylobacter jejuni* followed by *Campylobacter coli* is the most commonly reported species causing human infections.

Campylobacter spp. can be found in broilers at the farm, during processing, and in retail markets (Berrang et al. 2004). Intestinal contents and feces of broilers can harbor high numbers of *Campylobacter* bacteria (Jeffrey et al. 2001). During the course of slaughter and processing, there is potential for the alimentary tract to leak or rupture, spilling contents onto the skin or muscle of broiler carcasses. Once on the surface of a carcass, such contamination has the potential to persist through the remaining part of the carcass processing (Byrd et al. 2002) and potentially to cross-contaminate carcasses belonging to different flocks (Newell et al. 2001). The infective dose for humans of *Campylobacter* is very low, which means that even a very small number of *Campylobacter* cells in food samples represents a potential health hazard (Liu et al. 2006). A cross-contamination between poultry products and other foods, knives, kitchen utensils or cutting surfaces used during poultry meat preparation is an important way of human infection with campylobacters. Therefore, knowledge on occurrence and numbers of *Campylobacter* spp. on poultry products is of public health importance.

Aim of the present study. This study was designed to investigate the occurrence and numbers of *Campylobacter* spp. on broiler chicken wings and drumsticks at the retails level from three main poultry meat producers in Lithuania.

Materials and methods

Sampling plan. Samples of chicken wings and drumsticks were collected from three poultry meat producers by visiting randomly selected retail shops each week from March till October in 2009. During each visit at the shops samples of chicken wings and drumsticks of each producer were bought without giving a warning. A total 87 wings and 87 drumsticks samples were collected and tested for *Campylobacter* spp.

Isolation of *Campylobacter* spp. Collected samples were subjected to a whole sample rinse and the rinse decimal dilutions were cultured for *Campylobacter* spp. Briefly, each product was shaken manually with 100- ml buffered peptone water (BPW; Oxoid Ltd., Basingstoke, UK) in a technically clean plastic bag for 1 min, followed by 10- fold serial dilutions in BPW. Aliquots of 0.1 ml from the appropriate dilutions were plated on mCCDA selective medium. Inoculated mCCDA plates were incubated in microaerophilic atmosphere (85% nitrogen, 10% carbon dioxide and 5% oxygen) generated by Campygen (CN25; Oxoid Ltd., England) at 37 ^{0}C for 48 h. After incubation mCCDA plates were screened for presumptive colonies; these colonies were tested via phase-contrast microscopy for typical morphology and motility, counted and were further purified on blood agar plates (Blood Agar Base No. 2 (Liolfilchem, Italy) supplemented with 7% Laked horse blood and incubated at 37 ^{0}C for 48 h for 1- 2 days. From each sample up to 3 presumptive *Campylobacter* colonies were selected for the purification and further *Campylobacter* species identification. The purified isolates after growth on blood agar were subsequently stored at- 70 ^{0}C in BHI broth (BHI; Oxoid Ltd., Basingstoke, UK) with 20% of glycerol ("Stanlab", Poland).

A selective enrichment procedure was performed for each of the samples. For this procedure, 0,1 ml of the first dilution was placed in a tube containing a Bolton selective enrichment broth (CM0983; Oxoid Ltd., England) with Bolton broth selective supplement (SR0183E; Oxoid Ltd., England) and 5% Laked horse blood (SR0048; OxoidLtd., England). Enrichment tubes were incubated microaerobically at 42 ^{0}C for 24 h. After incubation, 10 µl of the broth was streaked onto plates with mCCDA agar. The identification and purification of *Campylobacter* isolates was further performed as described above.

DNA isolation. After growing the bacteria on blood agar plates, a loopful of bacterial culture was taken and suspended in 500 µl distilled water. The suspension was heated at 100 ^{0}C for 10 min and then centrifuged for 5 min at 14 000 rpm. Supernant was transferred into a new tube. Extracted DNA were used immediately for PCR amplification or stored at -20 ^{0}C until examination.

Detection and differentiation of thermophilic *Campylobacter* spp. by multiplex- PCR. *Campylobacter* isolates were identified at the species level by a slight modification of the method and primers described by Wang et al. (2002). Primers 23SF and 23SR created a 650 bp fragment which occurs in all *Campylobacter* spp. A 323-bp amplicon was generated for *C. jejuni* and a 126-bp amplicon was generated for *C. coli* by using a mix of oligonucleotide primers hybridizing to the *C. jejuni hipO* gene (primers CJF and CJR) or the *C. coli* g*lyA* gene (primers CCF and CCR).

Each PCR mixture contained 2.0 µl of a 2 mM deoxynucleoside triphosphate mixture, 2.5 µl of 10X reaction buffer, 2.5 µl of 25 mM $MgCl_2$, 0.25 µl of HotStart *Taq* DNA polymerase (MBI, Fermentas), 0.75 µl of a 100 µM primer mixture containing 23S rRNA, *Campylobacter jejuni* and *Campylobacter coli* primers, 2.5 µl of chromosomal DNA, and MiliQ water to a final volume of 25 µl. PCR products were analyzed by gel electrophoresis: 11 µl volume of each PCR product was loaded onto a 1.3% TopVision™ LM GQ Agarose gel (MBI, Fermentas) containing 0.05 µl/ml of ethidium bromide solution. The gel was visualized on an UV board. The GeneRuler^{TM}100 bp DNA Ladder (MBI, Fermentas) was used as the molecular size marker.

Results. Overall 174 samples from 3 main broiler producers in Lithuania were collected and examined. *Campylobacter* spp. were isolated from 81 (46.55%) out of 174 samples. Twenty nine samples out of 81 were confirmed as positive only after enrichment procedure. *C. jejuni* was identified in 69.12% and *C. coli* in 13.23%, both species together in 17.65% of samples. The quantification of *Campylobacter* spp. on broiler meat products showed that the mean number of *Campylobacter*

spp. detected on wings at the retail was 1.99 log10 CFU/ml of rinse and on drumsticks 2.11 log10 CFU/ml. The information on broiler meat contamination depending on broiler meat producer and product tested is presented in Table 1.

Table 1. **Occurrence and numbers of *Campylobacter* spp. on broiler wings and drumsticks from three different broiler meat producers in Lithuania**

Producer	No. of samples tested	Positive no. (% positive)	Mean; log10 CFU/ml	Product; mean log10 CFU/ml	
				Wings	Drumsticks
A	58	39 (67.24)	2.33	1.98	2.52
B	58	19 (32.76)	1.57	1.52	1.62
C	58	23 (39.66)	1.88	2.22	1.31
Average		81 (46.55)	2.04	1.99	2.11

Seasonal occurrence of *Campylobacter* bacteria on broiler meat products depending on month is presented in Figure 1. The occurrence was more frequent from July (70.83%) through September (66.66%) and the lowest in March (11.11%).

The median number of *Campylobacter* spp. on the contaminated drumstick and wings was 2.04 log10 CFU. Eighteen samples of wings and 17 samples of drumstick were contaminated with low numbers 1–2 log10 CFU/ml of *Campylobacter* spp., and higher numbers more then 2 log10 CFU/ml were determined only in 5 samples of wings and 6 samples of drumstick. The detailed information on the quantitative analysis is presented in Table 2.

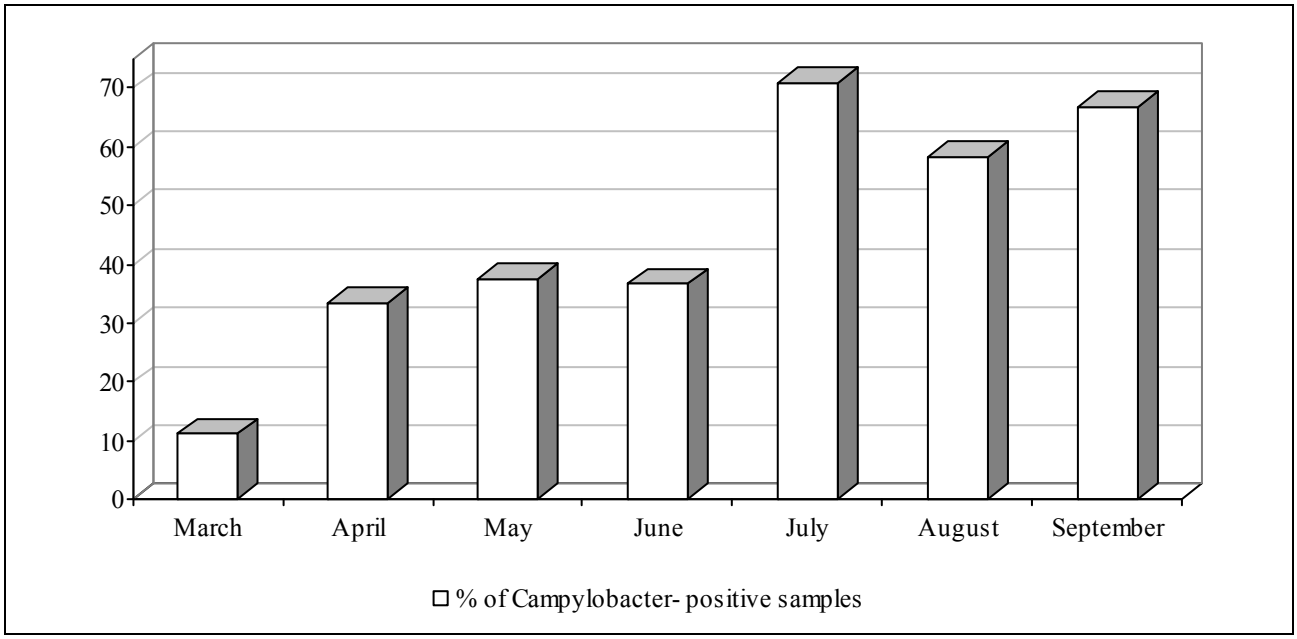

Fig. 1. **Seasonal occurrence of *Campylobacter* bacteria on broiler meat products from March to September 2009** (percentage of positive samples)

Table 2. **Exogenous contamination of chicken wings and drumstick with *Campylobacter* spp.**

Surface log10 CFU/ml	Wings (87)*		Drumstick (87)	
	Number of samples	Percentage of samples (%)	Number of samples	Percentage of samples (%)
Not found	45	51.72	48	55.17
< 1.0	19	19.54	16	13,79
1.0 - 2.0	18	20.69	17	19.54
2.0 – 3.0	5	5.75	5	5.75
> 3.0	-	-	1	1.15

Discussion. In the present study we investigated the occurrence and numbers of *Campylobacter* spp. on selected broiler products of the three main producers in Lithuania. Our results show that chicken wings and drumsticks at the retails level in Lithuania are frequently (46.55%) contaminated with *Campylobacter* spp. and it is the first data of this kind in Lithuania. The obtained data in Lithuania confirms that broiler chicken products are frequently contaminated with these bacteria. Similar results were reported by Ghafir et. al. (2007) where *Campylobacter* prevalence in broiler meat preparations from Belgian retail establishments was 49.4% and 44.9% in 2002 and 2003, respectively. Our study shows that chicken samples at retail in Lithuania are not so highly contaminated compare with the results reported in other countries. Recent raw chicken surveys in the United Kingdom have reported *Campylobacter* isolation rates ranging between 68 and 87% (Harrison et. al. 2001; Kramer et al. 2000; Meldrum et. al. 2004, Moore et. al. 2002). High prevalence of campylobacters on retail chicken parts (81.3%, 76.6% and 75.7% of products positive, respectively) were found in Italy, UK and France (Denis et al. 2001, Pezzotti et al. 2003, Sails et al. 2003). Such contamination of broiler meat at the retail level is not unexpected as previous studies showed high prevalence of Campylobacter spp. among broiler flocks. Results of study done by Kudirkiene et al (2010) showed, that among 42 broiler flocks examined in 1 year period, 31 flocks (73.8%) were positive for *C. jejuni* and 17 flocks (40.48%) for *C. coli*. The species identification of *Campylobacter* isolates obtained in our study showed that *C. jejuni* was found in the majority of cases (69.12%), followed by *C. coli* (13.23%) and these results are is comparable with previous studies done by Wedderkopp et al. (2000) and Reich et al. (2008).

C. jejuni and *C. coli* are generally considered to exist commensally in the gastro- intestinal tract of birds, particularly poultry, although there are usually no signs of disease in the animal itself (Altekruse et. al. 1998; Corry and Atabay, 2001). It is well known that poultry carcasses become extensively contaminated with *Campylobacter* from intestinal contents during slaughtering process and poultry meat is regularly contaminated with high loads of campylobacter (Berndtson et al. 1992). According to Stern and Robach (2003), *Campylobacter* levels found on carcasses may represent an important source, providing consumer exposure and a potential risk for campylobacter infection. Chicken wings were selected for testing in this study as they were identified as a particularly high- risk product group, since the high *Campylobacter* load in chicken wings could increase the probability of pathogen transfer to other surfaces through cross- contamination and inappropriate handling during meal preparation and cooking (Nauta et. al. 2007). During laboratory testing, it was notable that traces of feathers or feather shafts were commonly still connected to wing samples. *Campylobacter* originally associated with feathers might be transferred to the skin through the action of the picker's rubber fingers during mechanical feather removal in the slaughterhouse (Buhr et al. 2003). Feathers can be contaminated with feces during transport, and *Campylobacter* originally associated with feathers can be transferred to the skin during the plucking process (Berrang et al. 2000). Besides, the high *Campylobacter* count in chicken wings might be attributed to imperfect scalding, postscalding contamination, or due to the combination of both (Cason et al. 2004).

The seasonal changes on *Campylobacter* prevalence were seen during our study. The highest isolation rates we found from July through September and the lowest in March (Fig. 1). Such seasonal differences are related to lower temperature in September-March period as campylobacters are sensitive to lower temperatures. Similar findings were reported in a study conducted in the Netherlands, where the highest isolation rates were found from June through September (100%) and the lowest in March (50%) (Jacobs- Reitsma et. al. 1994). Also Willis and Murray (Willis et al. 1997) reported that *Campylobacter* shows a seasonal variation, with the highest contamination rate from May through October (87 up to 93%, respectively) and the lowest in December (7%) and January (33%).

The median count of 2.04 log10 CFU/ml of *Campylobacter* on the contaminated drumstick and wings was determined. No comparable studies were found in the literature were chicken wings and drumstick were examined. Usually the comparison between distinct studies is complicated due to different methodologies used for campylobacter enumeration on broiler meat surfaces. Though the study which quantified *Campylobacter* spp. on retail chicken legs (Luber et al. 2004) revealed higher numbers (median count of 4.01 log10 CFU/ml) on the surface of a contaminated legs portion comparing to our study results. According Luber et al., (2004), a percentage of 27.8 of legs carried between 4 log10 and 5 log10 CFU/ml, and 5.1% had a count of more than 5 log10 CFU/ml and these results are much higher in comparison to our study findings.

To our knowledge the occurrence and numbers of *Campylobacter* spp. on broiler meat at retail was not previously reported in Lithuania. Our study suggests that an improvement of control measures at farm and retail level is necessary to reduce the risk of infection with *Campylobacter* spp. for consumers. Furhter, public education of consumers on proper handling of poultry products and cooking may help to minimize the risk of infection with *Campylobacter* spp.

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