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Potravinarstvo Slovak Journal of Food Sciences
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Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 14, 2020, p. 815-820
https://doi.org/10.5219/1422
Received: 16 June 2020. Accepted: 20 July 2020.
Available online: 28 September 2020 at www.potravinarstvo.com

ISSN 1337-0960 (online)

PREVALENCE OF *CAMPYLOBACTER* SPP. IN A POULTRY AND PORK PROCESSING PLANTS

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ABSTRACT

The study aimed to investigate the prevalence of *Campylobacter* spp. in different stages of poultry and pork processing in the Central region of Russia. A total of 47 *Campylobacter* isolates were obtained from 107 samples from poultry processing plants (40.2%): 87.2% were identified as *Campylobacter jejuni*, whereas 12.8% were identified as *Campylobacter coli*. The prevalence of *Campylobacter* was significantly (p <0.05) higher after evisceration in the poultry processing plant. *Campylobacter* spp.was detected in 62.7% of the equipment and environmental samples. From positive samples of *Campylobacter spp.*, 84.3% of *Campylobacter jejuni*, and 15.7% *Campylobacter coli* were observed. A total of nine *Campylobacter* isolates were obtained from 116 samples from pork processing plants (7.8%): 33.3% of them were identified as *Campylobacter jejuni* whereas 66.7% were identified as *Campylobacter coli*. Splitting and evisceration were also critical in *Campylobacter* contamination. Almost all pork carcasses were *Campylobacter* positive, and all of them were identified as *Campylobacter coli*. The prevalence of positive *Campylobacter* samples in poultry processing plants was significantly (p <0.05) higher than in pork processing plants.

Keywords: *Campylobacter jejuni; Campylobacter coli*; poultry processing; pork processing

INTRODUCTION

Campylobacteriosis is still one of the most important infectious diseases that are likely to challenge global health in the years to come **(Kaakoush et al., 2015)**. According to the World Health Organization (WHO) reports, foodborne diseases, including Campylobacteriosis, are substantial: every year, almost one in 10 people fall ill and 33 million healthy life years are lost. *Campylobacter* is one of the four key global causes of diarrhoeal diseases **(WHO, 2020)**. The Centers for Disease Control and Prevention **(CDC, 2019)** estimates *Campylobacter* infection affects 1.5 million of the U.S. residents every year. Most cases are not part of recognized outbreaks, and more cases occur in summer than in winter **(EFSA and ECDC, 2019)**. The European Food Safety Authority (EFSA) reported that campylobacteriosis is the most common zoonotic disease in the EU. In 2018, member states reported 246,571 cases. The highest occurrence was detected in chicken meat (37.5%) and turkey meat (28.2%) **(EFSA and ECDC, 2019)**. Transmission typically occurs through the consumption of undercooked poultry or handling of raw poultry **(Altekruse et al., 1999; Blaser, 1997)**.

Studies have revealed that about 50% – 70% of human campylobacteriosis can be attributed to the consumption of poultry and poultry products **(Allos, 2001).** Various studies have demonstrated high levels of *Campylobacter* in the broilers, on the broiler carcasses, and retail chickens **(Zhao et al., 2001)**. Researchers have revealed this pathogen was detected in both dirty and clean transport crates, in scalding water, and on the de-feathering machine, and the working table at the end of the working day, but not at the beginning. After defeathering, *Campylobacter* spp. was detected in all of the sampled carcasses **(Perez-Arnedo and Gonzalez-Fandos, 2019)**. During slaughter, the main critical points for carcass contamination were identified as plucking, gutting, and final washing **(Facciolà et al., 2017)**. It was established that at low positive temperatures, *Campylobacter jejuni NCTC*11168 could remain viable in minced meat for at least seven days **(Bataeva and Sokolova, 2018)**.

However, in a study of goat and ovine milk in the Czech Republic, no *Campylobacter* bacteria were detected **(Bogdanovičová et al., 2015)**.

Campylobacter spp. survival was also investigated in the poultry industry before and after cleaning and disinfection. The fat removal machine, a gutting machine, a floor, a sink, a conveyor belt, shackles, and broiler meat were analyzed, and *C. jejuni* and *C. coli* were isolated. The results showed that the prevalence of *C. jejuni* and *C. coli* was 94.5% and 5.5%, respectively **(Sánchez et al., 2017)**. In one study, the detection of *Campylobacter* on carcasses was higher than that on cloacal swabs, which could

indicate cross-contamination during the slaughtering process **(Borges et al., 2020)**.

In some European countries, flock colonization of chickens with *Campylobacter* has a clear seasonal pattern, with the highest rates seen in the summer or autumn **(EFSA, 2010)**. The reasons for the seasonal variation are not fully understood but are likely to involve the frequency and nature of exposure of the flocks to *Campylobacter* spp. There is further evidence that climatic factors, such as temperature, correlate with both broiler flock and human infections **(Jorgensen et al., 2011)**.

Also, it has been reported that *Campylobacter* exhibits a cyclical pattern of contamination, where the level of contamination consistently increases and decreases depending on the season **(Hinton et al., 2004)**. Despite poultry are an important reservoir and source of human campylobacteriosis **(Hayama et al., 2011)**, the contribution of other sources, reservoirs, and transmission warrants further research. The predominant species in poultry is *C. jejuni*, whereas the predominant species of *Campylobacter* in pigs is *C. coli* **(Fosse et al., 2009; Horrocks et al., 2009; Varela et al., 2007)**. Authors also reported that control of this microorganism must rely on careful food processing and storage of pork, rather than an on-farm approach **(Varela et al., 2007)**.

Most human infections in the U.S. are associated with *C. jejuni*, whereas in Europe, a high incidence of human infection with *C. coli* is reported.

The authors reported that the sampling points with the greatest contamination rates were after evisceration, and contamination significantly decreased after chilling and washing **(Lee, et al., 2017)**.

Studies have shown that all processing plants sampled indicated a reduction in the *Campylobacter* populations along the processing line. Also, it was shown that proper cleaning of the equipment as well as a regular influx of freshwater, and using antimicrobials at the points of intervention during processing is crucial to preventing higher contamination **(Wideman et al., 2015; Berrang and Dickens, 2000)**.

Scientific hypothesis

This study was focused on the isolation of *Campylobacter* spp. from swabs of poultry and pork carcasses, and environmental swab samples from poultry and pork processing plants. The study aimed to investigate the prevalence of *Campylobacter* spp. in the processing of poultry and pork in Russian processing plants and to compare it with the European baseline data on Campylobacter prevalence.

MATERIAL AND METHODOLOGY

Poultry and pork processing plants in the Central region of Russia were selected. Swabs from poultry and pork carcasses and environmental swab samples from processing plants were selected as objects of the study. The following sampling points on the poultry processing line were selected: evisceration, processing and preparation, and packaging. The following sampling points on the pork processing line were selected: splitting and evisceration, removal of skin, deboning, and cutting.

Sampling

Environmental samples were taken using sterile sponges (3M TM, Saint Paul, 110 Minnesota, USA). Samples were transported at 4 °C to the laboratory and processed within 24 h.

Detection of *Campylobacter* spp.

The isolation of *Campylobacter* spp. was performed according to **ISO 10272-1 (2017)**. Environmental samples were performed according to **ISO 18593 (2018)**. They were taken using sterile sponges from 100 cm^2 and homogenized in 100 mL of Bolton broth (Merck, Germany). Swabs of poultry and pork carcasses were homogenized for 20 s with 225 mL of Bolton broth. The samples were incubated at 41.5 °C for 44 h under a microaerobic atmosphere. *Campylobacter* isolation was done on modified charcoal cefoperazone deoxycholate agar (mCCDA) (Merck, Germany) and selective agar Preston under microaerobic conditions at 41.5 °C for 44 h. Confirmation of presumptive colonies was performed according to the **ISO 10272-1 (2017)** principles – typical colonies were seeded on blood agar (Oxoid, UK) and incubated at 41.5 °C for 24 h and then confirmed using biochemical tests (Oxoid, UK).

Statistical analysis

StatPlus 6.2.2.0 Software (AnalystSoft) was used. Tukey's test for the comparison of means was performed using the same program. The significance level was defined at $p < 0.05$.

RESULTS AND DISCUSSION

Presence of Campylobacter spp. in environmental samples and poultry carcasses at various stages of poultry processing.

A total of 47 *Campylobacter* isolates were obtained from 107 environmental samples and poultry carcasses (40.2%): 87.2% were identified as *C. jejuni* whereas 12.8% were identified as *C. coli* (Figure 1).

Table 1 shows the presence of *Campylobacter* at different stages of poultry processing. After evisceration, *Campylobacter* spp. was detected in 62.7% of the equipment and environmental samples. From positive samples of *Campylobacter* spp. 84.3% of *C. jejuni* and 15.7% *C. coli* was observed. The predominance of *C. jejuni* over *C. coli* has been shown by other authors **(Sánchez et al., 2017)**. In that study, the abundances of *C. jejuni* and *C. coli* were 94.5% and 5.5%, respectively. These results confirmed those reported by **Lee et al. (2017)** that the greatest contamination rates were after evisceration. According to **Facciolà et al. (2017)** during slaughter, the main critical points for poultry carcass contamination were identified by plucking, gutting, and final washing. Other authors described slaughtering and evisceration as critical points of *Campylobacter* contamination **(Gruntar et al., 2015; Sasaki et al., 2013)**.

Campylobacter spp. was not detected after deboning and cutting, but it was found after packaging. The *Campylobacter* spp. isolated during packaging was identified as *C. jejuni*.

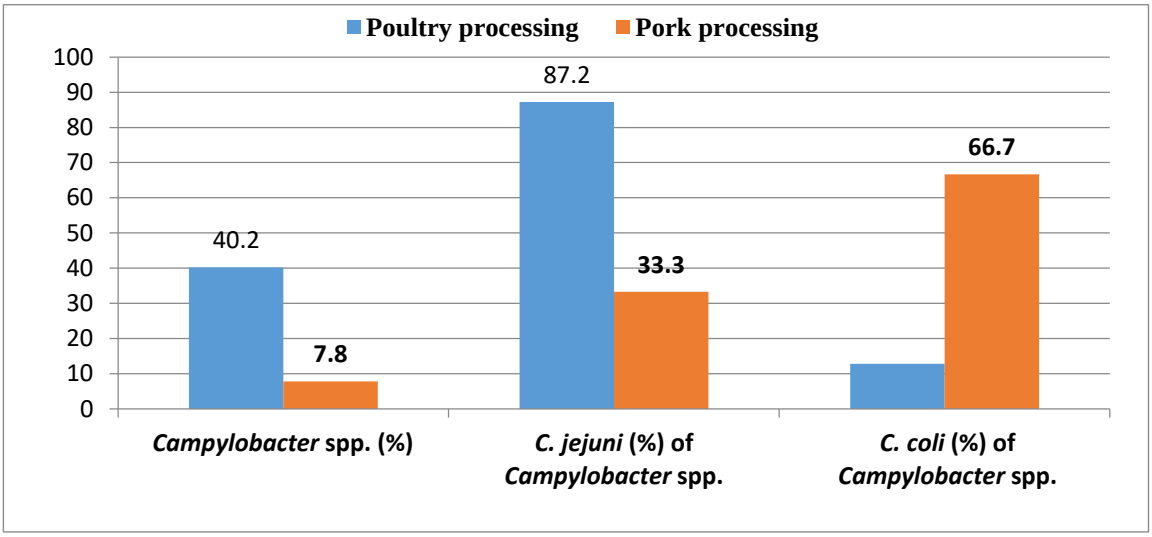

Figure 1 Prevalence of Campylobacter in poultry and pork processing plants.

Table 1 Presence of *Campylobacter spp.* in environmental samples and poultry carcasses at various stages of poultry processing.

Sampling location/Sample	*Campylobacter*/Total (%)	*C. jejuni*/Total positives (%)	*C. coli*/Total positives (%)
Evisceration	32/51 (62.7)	27/32 (84.3)	5/32 (15.7)
Bonning and cutting	0/9 (0.0)	0/0 (0.0)	0/0 (0.0)
Packaging	1/9 (11.0)	1/1 (100.0)	0/1 (0.0)
Poultry carcasses (total):	14/38 (36.8)	13/14 (93.0)	1/14 (7.0)
-cloaca	6/12 (50.0)	6/6 (100.0)	0/6 (0.0)
-legs	3/12 (25.0)	2/3 (67.0)	1/3 (33.0)
-carcasses	4/12 (33.0)	4/4 (100.0)	0/4 (0.0)
-neck	1/12 (8.3)	1/1 (100.0)	0/1 (0.0)

It is also an important contamination point due to the possible intestinal ruptures that can occur during the mechanical removal of the intestines **(Perez-Arnedo and Gonzalez-Fandos, 2019)**. Moreover, 50% of the investigated cloacal swabs samples were *Campylobacter* positive. These two stages can be related to each other and can cause cross-contamination of carcasses. Also, 5 mg of caecal content can increase the number of Campylobacter on eviscerated broiler carcasses **(Berrang et al., 2004)**. These findings support the idea of cross-contamination from contaminated equipment and work surfaces to carcass. Studies are confirming the genetic identity of the strains contaminating slaughterhouse equipment and meat products **(Elvers et al., 2011; Prachantasena et al., 2016)**.

Thirty-three percent of the investigated carcasses were *Campylobacter* positive. All *Campylobacte*r positive samples from cloacal swabs, carcasses, and necks were identified as *C. jejuni*.

However, in our research, the prevalence of *Campylobacter* was significantly (p <0.05) higher after evisceration than in carcasses. It is very important to decrease *Campylobacter* prevalence in poultry meat, because although *Campylobacter* spp. do not replicate in food **(Corry and Atabay, 2001)**, a low dose can cause an infection **(Vidal et al., 2014)**.

C. coli was detected in five environmental samples after evisceration and in the leg of one poultry sample.

Presence of Campylobacter spp. in environmental samples and pork carcasses at various stages of pork processing.

A total of nine *Campylobacter* isolates were obtained from 116 environmental samples and pork carcasses (7.8%): 33.3% of them were identified as *C. jejuni* whereas 66.7% were identified as *C. coli* (Figure 1). As reported in previous studies, *C. jejuni* prevailed in the poultry farm compared to the lower presence of *C. coli* **(Pepe et al., 2009; Peyrat et al., 2008; Wieczorek et al., 2015)**.

Table 2 shows the presence of *Campylobacter* at different stages of pork processing. After splitting and evisceration, *Campylobacter* spp. was detected in 7.4% of the equipment and environmental samples. A significant difference (p <0.05) in positive *Campylobacter* samples was found between poultry and pork evisceration. The prevalence of positive *Campylobacter* samples in poultry processing was significantly (p <0.05) higher than in pork processing. From two positive samples of *Campylobacter* spp, *C. jejuni* was observed. Environmental and equipment samples after removal of skin, deboning, and cutting were investigated. One of them was identified as *C. jejuni*, another one as *C. coli.*

Pork carcasses (neck, leg, belly, skin) were also investigated for the prevalence of *Campylobacter* spp. Almost all pork carcasses were *Campylobacter* positive, and all of them were identified as *C. coli*.

Table 2 Presence of Campylobacter spp. in environmental samples and pork carcasses at various stages of pork processing.

Sampling location/Sample	*Campylobacter*/Total (%)	*C. jejuni*/Total positives (%)	*C. coli*/Total positives (%)
Splitting and evisceration	2/27 (7.4)	2/2 (100)	0/2 (0.0)
Removal of skin	1/32 (3.1)	1/1 (100.0)	0/1 (0.0)
Bonning and cutting	1/21 (4.8)	0/1 (0.0)	1/1 (100.0)
Pork carcasses (total):	5/36 (13.9)	0/5 (0.0)	5/5 (100.0)
-neck	2/9 (22.2)	0/2 (0.0)	2/2 (100.0)
-leg	0/9 (0.0)	0/0 (0.0)	0/0 (0.0)
-belly	2/9 (22.2)	0/2 (0.0)	2/2 (100.0)
-skin	1/9 (11.1)	0/1 (0.0)	1/1 (100.0)

Our results confirm those reported by others, who found the predominant species of *Campylobacter* in pigs was *C. coli* **(Fosse et al., 2009, Horrocks et al., 2009; Varela et al., 2007)**. While the reservoirs of *Campylobacter* are recognised as both poultry and pigs **(Quintana-Hayashi and Thakur, 2012)**, *C. coli* is the main species found in pigs **(Avrain et al., 2004)**. Authors also reported that control of this microorganism must rely on careful food processing and storage of pork **(Varela et al., 2007)**. A factor that is associated with an increased risk of *Campylobacter* in pork is a high level of contamination in farms. Bacteriological study results showed that 77% of the piglets and 100% of the fattening pigs were infected with high levels of contamination, but *Campylobacter* was not detected after deboning **(Minvielle et al. 2007)**. The authors also note the importance of animal selection, transportation to the slaughterhouse, and time spent in the slaughterhouse **(Hald, Sommer and Skovgård ,2007)**.

The application of strict biosecurity measure proved to be effective in preventing the *Campylobacter spp.* contamination. There are: cleaning and disinfection of the plant equipment; a control of the entry of persons, birds, rodents or other animals; an insect control; water control; waste control **(Hansson et al., 2007; Guerin et al., 2007; Nesbit et al., 2001)**.

It was previously reported that survival during storage and under stress factors, such as microaerophilic conditions, *Campylobacter* in food products could be aerotolerant. Interestingly, a greater prevalence of aerotolerant strains (80%) was found among *C. coli* isolates as compared to *C. jejuni* isolates (6%); these strains were previously isolated from retail chicken meat, chicken livers, chicken gizzards, turkey, pork, and beef liver samples **(Karki et al., 2018)**.

Many studies describe the antibiotic resistance of *Campylobacter* strains **(Noormohamed and Fakhr, 2014)**. The increasing trend of antimicrobial resistance among *Campylobacter* strains indicates a high risk of new outbreaks **(Geissler et al., 2017)**.

Further studies are needed to investigate the antimicrobial resistance profile and aerotolerance of isolated *Campylobacter* strains. Potential approaches for the control of *Campylobacter* in processing poultry and pork plants are also necessary.

CONCLUSION

Campylobacter prevalence was estimated at poultry and pork processing plants in the Central Region of Russia. A total of 47 Campylobacter isolates were obtained from 107 samples of poultry processing (40.2%): 87.2% were identified as *C. jejuni*, whereas 12.8 % were identified as *C. coli*. The prevalence of *Campylobacter* was significantly (p <0.05) higher after evisceration in poultry processing plants: *Campylobacter spp.* was detected in 62.7% of the equipment and environmental samples. Of the positive samples of *Campylobacter* spp., 84.3% of *C. jejuni* and 15.7% *C. coli* were observed. A total of nine *Campylobacter* isolates were obtained from 116 samples of pork processing (7.8%): 33.3% of them were identified as *C. jejuni*, whereas 66.7% were identified as *C. coli*. Splitting and evisceration were a critical point of *Campylobacter* contamination. Almost all pork carcasses were *Campylobacter* positive, and all of them were identified as *C. coli*. The prevalence of positive *Campylobacter* samples in poultry processing was significantly (p <0.05) higher than in pork processing. The prevalence of *Campylobacter* was significantly (p <0.05) higher after evisceration in poultry processing plants: *Campylobacter spp.* was detected in 62.7% of the equipment and environmental samples. Among the positive samples of *Campylobacter* spp., 84.3% of *C. jejuni* and 15.7% *C. coli* was observed.

Further studies are needed to investigate the antimicrobial resistance profile and aerotolerance of isolated *Campylobacter* strains. Potential approaches for the control of *Campylobacter* in processing poultry and pork plants are also necessary.

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