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 (Facciòlala et al., 2017). It was established that at low positive temperatures, Campylobacter jejuni NCTC11168 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 cm² 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 ceftazidime 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 Faccioli 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.
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 Campylobacter 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.

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

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

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 Skovgaard, 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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Prevalence of *Campylobacter spp.*, *Escherichia coli* and *Salmonella* serovars in retail chicken, turkey, pork and 

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