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THERMOPHILIC *CAMPYLOBACTER* SPP.
IN POULTRY MEAT PRODUCTION

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Abstract

Thermophilic *Campylobacter* spp. are the leading cause of zoonotic enteric disease in Europe and USA. In Serbia, it has an upward trend in human population. The disease is usually indirectly transmitted to humans through the consumption of food contaminated by the faeces of infected animals. The aim of this paper was to analyze data on the prevalence of *Campylobacter* spp. in poultry meat production chain and the risk for the development of the disease in humans. The *Campylobacter jejuni/coli* was identified at farm level in 73.3% of poultry, 66.6% calves and 58.3% pig samples of already ill or suspected cases. Clinical manifestation of the disease in birds can be expected if an additional immunosuppressive factor is present. Artificial infection of healthy chickens with 6.77 log cfu *C. jejuni* per chicken on day 21st of life leads to 5.26 log cfu/g faeces after only five days with a tendency to decrease during the next 18 days. Although chilling and freezing may significantly reduce *Campylobacter* contamination of carcasses, it cannot completely eliminate the initial contamination. According to our experimental results the prevalence of *Campylobacter* contaminated chickens from positive flock appears to drop from 100% live birds (with 3.02 log cfu/g faeces) to 50% of chicken carcasses. Contamination of the carcasses depends on initial contamination of live birds, good hygiene practices and good manufacturing practices. Therefore, high variability in contamination of carcasses can be considered; prevalence range from 11.43 to 90.00% of carcases was established in various slaughterhouses. At retail, *Campylobac-*

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ter was detected in 18.8% poultry meat samples and 10.0% samples of other meat types. *Campylobacter* is frequently found in the entire production chain of poultry meat and represents high risk for consumers' health.

Key words: *Campylobacter*, farm, slaughterhouse, retail

TERMOFILNE *CAMPYLOBACTER* VRSTE U LANCU PROIZVODNJE PILEĆEG MESA

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Kratak sadržaj

Termofilne *Campylobacter* vrste su jedan od vodećih uzročnika alimentarnih oboljenja u Evropi i USA. U Srbiji postoji rastući trend incidence u humanoj populaciji. Oboljenje se prenosi na čoveka putem hrane koje je kontaminirana fecesom inficiranih životinja. Cilj rada je analiziranje raširenosti *Campylobacter* spp. u lancu proizvodnje mesa živine i utvrđivanje značaja rizika za zdravlje ljudi. *Campylobacter jejuni/coli* je identifikovan na nivou farme u 73.3% uzoraka poreklom od živine, 66.6% teladi i 58.3% svinja. Uzorci u ovom istraživanju su poticali od bolesnih ili sumnjivih životinja. Klinička manifestacija oboljenja kod živine se očekuje samo u slučaju prisustva dodatnih, imunosupresivnih faktora. Veštačkom infekcijom zdravih brojlera sa 6.77 log cfu *C. jejuni* 21. dana života, već nakon pet dana dolazi do izlučivanja 5.26 log cfu/g fecesa sa tendencijom opadanja tokom narednih 18 dana. Iako hlađenje i zmrzavanje mogu značajno redukovati kontaminaciju živinskih trupova, nije moguće u potpunosti eliminisati početnu kontaminaciju. Prema našim eksperimentalnim podacima prevalenca *Campylobacter* kod veštački inficiranih pilića opada sa 100% živih jedinki (3.02 log cfu/g fecesa) na 50% klanično obrađenih trupova. Kontaminacija trupova zavisi od početne infekcije živih jedinki i dobre higijenske i dobre proizvodne prakse primenjene u klanici. Stoga nije neobično da postoji visoka varijabilnost u kontaminaciji trupova

poreklom iz različitih klanica, od 11.43 do 90.00%. U prometu prisustvo *Campylobacter* je detektovano kod 18.8% uzorka živinskom mesa i 10.0% uzorka ostalih vrsta mesa. *Campylobacter* ima visoku prevalencu u celom lancu proizvodnje živinskog mesa od farme, preko klanice pa sve do mesa u prometu i predstavlja značajan rizik za zdravlje potrošača.

Ključne reči: *Campylobacter*, farma, klanica, promet

INTRODUCTION

Campylobacter spp. are microaerophilic, Gram-negative, curved and motile rods, which commonly cannot grow below 30°C; however, some low metabolic activity is detectable at 40°C (EFSA, 2010). The most important foodborne *Campylobacter* species are thermophilic *C. jejuni* and *C. coli*. *C. jejuni* can be found in the intestines of a range of wild and domestic animals, especially birds. Its prevalence in birds could be attributed to the optimal growth temperature being 42°C. *C. coli* can often be identified in the intestines of pigs. In the environment, the organism is isolated from the water, dust, soil, air and also in fish and vegetables (EFSA, 2010). Virulence factors of *Campylobacter* include motility, chemotaxis, adherence and invasion (Bolton 2015).

Thermophilic non-foodborne *Campylobacter* species are common causative agents of abortions in sheep and cattle and, occasionally, in other animal species. Thermophilic foodborne species can cause diarrhoea in animals and hepatitis in birds. *Campylobacter jejuni/coli* often colonize the alimentary tract of poultry and other domestic livestock, yet without development of diseases symptoms. The organisms can commonly be found in the caecum, colon and cloaca, colonizing the crypt and villus region, and are present in the mucus but not in the epithelium. Mucus is the environment that provides optimal *oxygen* level and improved motility of the bacteria (Mead, 2002). *Campylobacter jejuni/coli* are much more frequently detected in the caecum and rectum (70.0%) than in the reproductive system (uterus and magnum) (6.7%) of poultry (Stojanov et al., 2008).

CAMPYLOBACTER SPP. IN POULTRY FARMING

Campylobacter is present in farm surrounding environment, including the soil, water sources, dust, building surfaces, and the air (Ellis-Iversen et al., 2012). Major contamination sources include the environment, domestic and wild animals, water, partial depopulation (thinning) and carry over from

previous flocks (Barrios et al., 2006; Newell et al., 2011). The prevalence of *Campylobacter* positive flocks in Europe greatly differs between the countries and ranges between 5 and 90% depending on the geographic location, climatic differences, biosecurity measures as well as different research methodologies (Berndtson et al., 1996a,b).

If *Campylobacter* is introduced into the flock at an early phase of breeding, the colonization will most likely persist in all birds until slaughter, and the flock prevalence can reach even 100%. *Campylobacter* infection spreads within the flock via bird-to-bird transmission route. Such a rapid spread is a result of high expression level (8-9 log cfu/g faeces), coprophagia as well as contamination of drinking water and feeders (Thibodeau et al., 2015). The spread of *Campylobacter* is mediated also by the presence of flies, mice, and farm workers as the mechanical transmission vectors. Low flock prevalence of *Campylobacter* indicates recent colonization (Evans and Sayers, 2000).

Colonization occurs most commonly in birds at the age of 2-4 (van Gerwe et al., 2009). Despite extensive colonization, clinical symptoms in poultry are rare and unspecific and include watery diarrhoea often in the absence of pathoanatomical changes in the jejunal mucosa and the caecum. The intestinal bloating and distension consequent to the accumulation of watery and mucous contents are also diagnosed (Evans and Sayers, 2000). Macroscopic necrotic changes are visible in the liver of poultry (Lemos et al., 2015). Diarrhoea commonly occurs some 6 hours post infection, and can persist for up to 10 days. Clinical signs are greatly determined by the amount and virulence of the individual strain of *C. jejuni*, stress factors and immunosuppression (Evans and Sayers, 2000). Our research revealed that clinically manifest campylobacteriosis occurred in experimentally infected chickens with both *C. jejuni* and *Salmonella* Enteritidis or *C. jejuni* with simultaneous administration of live D78 Gumboro vaccine. Major clinical symptom was watery diarrhoea that varied in intensity between birds. Diarrhoea did not occur in chickens infected with only *C. jejuni* or only *Salmonella* Enteritidis. The administration of immunosuppressive agents induced clinically manifested signs associated with the occurrence of red or yellow spots on the liver (Stojanov et al., 2008). Immunosuppressive agents such as dexamethasone, ochratoxin A and secondary bacterial infections also promote clinical signs of campylobacteriosis (Stojanov et al., 2011).

In chicken categories older than three weeks, the infection with *C. jejuni* strains results in intestinal colonization, yet not in the clinical manifestations of the disease (Petrović et al., 2008; Petrović et al., 2012). Chickens inoculated with *C. jejuni* at the beginning of the fourth week of age (21st day of life) had

an average *C. jejuni* count of 5.26 log cfu/g on day 5 post-infection. A trend of *C. jejuni* count is displayed in Figure 1. after an initial growing tendency during first five days after infection, a continuous decrease has been observed during the period between day 11 and day 18 post-infection (from 4.97 to 3.02 log cfu/g) followed by an increase in *C. jejuni* count to 4.95 log cfu/g (day 28 post-infection).
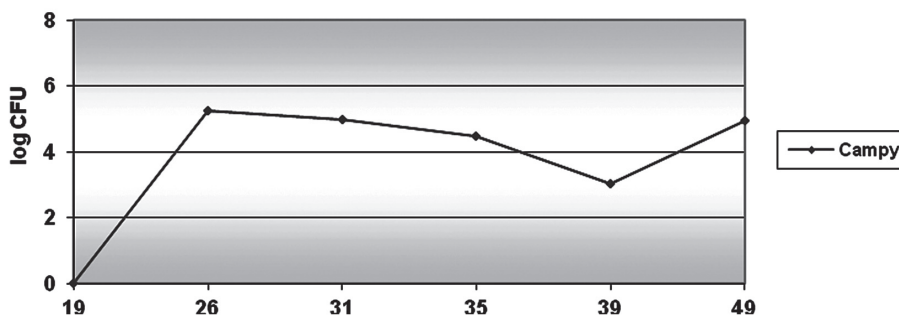

Figure 1. Average total count of *C. jejuni* in infected chickens (Petrović et al., 2012)

Surrounding environment including air distribution in the facility, open doors and windows, improper footwear, two or more workers in charge for the facility, presence of rodents and insects, two or more housing units at the farm, vicinity of farms with other domestic livestock, feed storage outside the facility and on-farm storage of broiler carcasses before distribution can represent important risk factors for the development of infection in broilers. Feedstuffs are not a suitable medium for multiplication of *Campylobacter* organisms, so drinking water remains far more important risk factor (Sibanda et al., 2018).

In cases when there are several units for flock accommodation at the same farm, *Campylobacter* infection can be identified in some flocks, while other ones remain *Campylobacter*-free. The susceptibility to colonization with *Campylobacter* strains increases with the age of the birds and flock size (Berndtson, 1996b). Gibbens et al. (2001) reported reduction of facility contamination by over 50% after application of relevant disinfection protocols. Poultry transportation equipment and cages are also potential sources of *Campylobacter*. Transportation negatively affects the poultry population and increases contamination due to the exposure to other birds' faeces (which is more likely to be watery due to stress conditions) (Dogan et al., 2019). The transmission of *Campylobacter* spp. across the farm occurs rapidly and the infection spreads flock-wide within only few days, which supports the theory that if the infection is present, every single bird in the flock is affected (Berndtson 1996b).

CAMPYLOBACTER SPP. IN SLAUGHTERHOUSES

Dominant route of *Campylobacter* contamination of poultry carcasses in a slaughterhouse is the rupture of intestines and consequent leakage and spread of the faeces. Another source is improper hygiene practice (cleaning and disinfection) in the facility (Hansson et al., 2005; Peryat et al., 2008). Carcass contamination is substantially influenced by biological properties of *Campylobacter*. Though microaerophilic and thus difficult to grow under common conditions in the food production chain, *Campylobacter* survives the acidative and oxidative stress and modified atmospheric packaging. This strongly indicates its high capability of adapting to unfavourable environmental conditions and surviving food processing procedures. Biological properties enabling the adaptability of this organism to stress include antioxidant defence, ability to enter to viable but not countable state (VBNC state), selection of virulent strains, antimicrobial resistance genes transfer and wide genetic variability (Meredith et al., 2014; Bolton 2015; Gomes et al., 2018).

Campylobacter contamination at slaughterhouse is closely related with the colonization status of broilers during rearing. Colonized chickens carry large amounts of *Campylobacter* in the caecum (5-8 log cfu) and intestines. Such chickens shed *Campylobacter* in the faeces during transportation and spread it into the environment (Dogan et al., 2019). Flock prevalence rate and *Campylobacter* concentration in the caecum along with the hygienic standards applied during processing strongly influence the amount of the pathogens in final poultry products and hence the exposure of the consumers to thermophilic *Campylobacter* species from poultry meat.

Major points of potential contamination in the slaughterhouse include picking, evisceration and some chilling processes. Leaking of faecal content during picking and evisceration process leads to carcass contamination; even very small amounts of the faeces can significantly elevate the *Campylobacter* counts on the carcasses. Immersion chilling with chlorinated water reduces contamination but without chlorine the contamination increases (Guerin et al., 2010; Dogan et al., 2019).

Contamination of carcasses could be reduced by scalding, washing and cold storage. During scalding, a part of *Campylobacter* organisms is washed out of the carcass surface. However, scalding water easily becomes contaminated with faecal contents and dust, thus causing recontamination of the carcass with certain amount of pathogens if scalding technology is not properly applied. The temperature of scalding water does not significantly influence the *Campylobacter* counts. Washing substantially reduces the concentration of *Campylobacter*

(90%), while airflow-chilling reduces *Campylobacter* counts depending on the temperature and humidity (Guerin et al., 2010; Dogan et al., 2019). *Campylobacter* contamination can be detected on all parts of broiler carcass neck skin, visceral cavity, even the region under the skin. The number of *Campylobacter* on the carcass commonly ranges around 2-3 log cfu/cm². Dry environment as well as the storage at 4°C or freezing result in the decrease of *Campylobacter* counts. Freezing at 18°C during 10 and 21 days will reduce the number of *Campylobacter* for 90% and 99%, respectively. However, there are reports on *Campylobacter* isolation from broiler carcasses even after 83 weeks of freezing. Even though current processing procedures reduce the *Campylobacter* count, the organism can often be found in final products at the range 1-4 log/cm². Cross-contamination at the slaughterhouse is considered possible yet limited, having in mind low concentration of *Campylobacter* on cross-contaminated carcasses as related to those originating from poultry that has previously been colonized with *Campylobacter*. Transmission of *Campylobacter* from cross-contaminated meat to the final products during processing is very unlikely, as well as the probability for consequent human infection and disease (EFSA, 2010).

The rate of carcass contamination at the slaughterhouse varies among poultry originating from *Campylobacter* colonized flocks. At certain production stages, the count of *Campylobacter* on the carcass can increase or decrease, and there are reports about complete elimination of all *Campylobacter* so the carcasses were free from *Campylobacter*. Various technological procedures applied at the slaughterhouses can affect cross-contamination and contamination of carcasses of poultry originating from *Campylobacter*-free flocks (Rosenquist et al., 2003). Slaughtering of flocks with 100% of infected birds results in 50% contaminated carcasses (Petrović et al., 2008). The prevalence of contamination significantly varies between individual slaughterhouses (Table 1).

Table 1. Occurrence of thermophilic *Campylobacter* spp. in poultry samples (Petrović et al., 2008)

Occurrence of *Campylobacter* spp. (%)	Abattoir mark						
	A	B	C	D	E	F	G
liver	40.00	5.00	8.56	6.00	34.28	2.86	5.71
carcasses	90.00	14.28	51.43	20.01	68.57	11.43	31.43

CAMPYLOBACTER SPP. AT RETAIL

The presence of thermophilic *Campylobacter* in meat on the market varies depending on the product type the highest risk is associated with whole poultry carcass, somewhat lower prevalence is evident in skin-off whole pieces. Minced meat is considered low-risk product because of beneficial effects of spices and oxidative stress as well as the range of poultry meat products (such as sausages, etc.) (Simone et al., 2017). In Serbian market, poultry meat is available as chilled (+4°C) or frozen (-20°C) product. Chilling does not significantly affect the survival of *Campylobacter*, whereas freezing reduces contamination (Reiersen et al., 2001). *Campylobacter* has been identified in 18.8% poultry meat samples and 10.0% of samples of other meat samples in a retail chain (Trajković et al., 2007).

Campylobacteriosis is the most commonly reported gastrointestinal human disease in the EU/EEA (EFSA and ECDC, 2018). Most common sources of human disease are undercooked or improperly heat treated meat, unpasteurized milk, dairy products and contaminated water. Some half of all cases of human campylobacteriosis in the USA is considered to be associated with consumption of poultry meat. After the incident with dioxin contamination in Belgium in 1999, poultry meat and relevant products were withdrawn from the market, which resulted in a 40% drop of the incidence of human campylobacteriosis. Grilling meat is at particular risk of infection development due to easy transmission of bacteria from raw meat to the hands and thus to other food while handling (Jorgensen, 2002). The risk of campylobacteriosis associated with consumption of pork and beef meat is considered relatively law; however, undertreated offal products still pose substantial risk. Important sources of human campylobacteriosis include drinking water and fresh products contaminated with campylobacteria from irrigation water. The presence of *Campylobacter spp.* in drinking and irrigation water is due to faecal contamination (EFSA, 2010).

CONTROL MEASURES

Qualitative risk analysis revealed that decrease in flock contamination level by 3 log cfu does not lead to significant decrease in the number of contaminated carcasses processed at the slaughterhouse; however, the *Campylobacter* count on the carcasses was significantly reduced (Reiersen et al., 2001). Quantitative risk analysis proved that even law reduction of *Campylobacter* spp. count in poultry faeces (2 log) decreases the incidence of human infection 30 times (Rosenquist et al., 2018) suggesting that control, that is, reduction of

Campylobacter in poultry faeces at the farms is a crucial measure for the prevention of human infections. Quantitative risk analysis also revealed a linear relationship between flock prevalence and number of contaminated carcasses after processing in the slaughterhouse, thus, compliance to strict hygienic barriers is the only effective approach to reducing the flock prevalence of *Campylobacter* (Reiersen et al., 2001).

Reduction of *Campylobacter* count on carcass surface can be accomplished by increasing scalding temperature, improving evisceration techniques, usage of large amounts of water across the entire production line, increasing the airflow at chilling and introducing relevant disinfection practices. Prevention of cross-contamination during transport of live chickens is of vital importance, also. However, all the aforementioned measures are of lesser influence on the decrease in rate of human infections as compared to the reduction of the incidence of *Campylobacter* in the flock.

CONCLUSION

Campylobacteriosis is one of the most important foodborne diseases mostly associated with consumption of contaminated poultry meat. In order to reduce the number of disease episodes, relevant measures should be applied across the entire poultry meat production chain and especially at poultry farms. Strict compliance with relevant biosecurity measures prevents the introduction of the pathogen into the production process at the farm. Adequate hygiene and sanitation practices in the slaughterhouses can prevent cross-contamination and decrease the contamination level. Ensuring of cold chain supply of poultry meat also contributes to an effective disease control. Successful protection of human population from alimentary diseases is closely associated with a range of preventive activities across the entire meat production chain and responsibility of each individual participant of this production chain.

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Author's contributions:

JP and RR made contributions to conception and design of the article, involved in data collection and drafting the manuscript. IS contributed with data about *Campylobacter* at farm level and manifestation of clinical campylobac-

teriosis. JP contributed with data about *Campylobacter* dynamics in artificially infected poultry and data about slaughterhouse prevalence. VG and JL contributed with data about *Campylobacter* prevalence at retail level. SM revised the manuscript critically and together with JP prepared the final draft of the manuscript. All authors read and approved the final manuscript.

Competing interest

Authors declared no conflict of interests regarding the present paper.

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