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Prevalence and factors affecting the presence of *Campylobacter* spp. in broiler carcasses in Bulgaria

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Abstract: In an 11-month study, 292 samples from 13 slaughterhouses in Bulgaria were investigated for the presence of *Campylobacter* spp., and some factors affecting *Campylobacter* prevalence were analyzed. The study revealed that 44.9% of the samples were positive for *Campylobacter* spp. The main species detected were *C. coli* and *C. jejuni*. There was also one confirmed *C. lari* isolate. The *C. coli* strains prevailed over *C. jejuni* at 61.8% and 37.4%, respectively. A seasonal variation in the *Campylobacter* spp. presence in slaughter broiler carcasses was found with a predominance of positive isolates during June and July 2008. The conventional type of rearing was responsible for higher *Campylobacter* contamination (52.2%) of broiler carcasses compared to free-range rearing (38.5%). The impact of the chilling technology (spray, immersion, or air-chilling) on the *Campylobacter* presence in broiler carcasses was also analyzed.

Key words: *Campylobacter* spp., broilers, *Campylobacter* prevalence, type of rearing

Introduction

The genus *Campylobacter* belongs to the family *Campylobacteriaceae*. It includes gram-negative, spiral-shaped bacteria, most of which grow in microaerobic conditions between 30 and 42 °C, but some have good growth capabilities in aerobic and anaerobic atmospheres out of the optimum (1). Some *Campylobacter* spp. are commensals and colonize the gastrointestinal tracts of many host species, while others are associated with different diseases in animals and humans (2,3). In the past few decades, *Campylobacter jejuni* and *Campylobacter coli* have been recognized as foodborne pathogens of major public health significance (4-6). Both species cause gastroenteritis in humans, as well as complications such as the Guillain-Barré syndrome and reactive arthritis (4).

According to the latest European Food Safety Authority (EFSA) data, enteric infections caused by *Campylobacter* spp. are the most frequently reported zoonoses among the human population in the European Union, with an incidence rate of 50 cases per 100,000 in over 17 countries (7-9). Many reports from various countries confirm the significant role of *Campylobacter* spp. in human gastroenteritis (10-12). The main source of human campylobacteriosis is poultry meat and poultry products (2,7,11).

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Using various molecular techniques, many authors (12-15) have proven the existence of similar genetic patterns between human and poultry *Campylobacter* strains. Their studies have also described the circulation of *Campylobacter* in the food chain from the primary production stage to the consumers. Taremi et al. (16) revealed the importance of similar studies concerning the distribution and diversity of *Campylobacter* spp. in poultry slaughterhouses, one of the main sources of *Campylobacter* in the food chain. There are considerable differences in the slaughter procedures that could influence the contamination level and cross-contamination of the broiler carcasses. In poultry slaughterhouses, the technological stage of greatest significance for the final broiler carcass *Campylobacter* spp. burden is the type of chilling (immersion, spray, or air).

The aim of the current study was to evaluate the prevalence of *Campylobacter* spp. in broiler carcasses from 13 slaughterhouses in Bulgaria during an 11-month period and the influence of rearing type, as well as the different carcass chilling technologies, on the contamination rate of *Campylobacter*.

Materials and methods

Preparation of samples

Investigated were 292 chilled broiler carcasses, representing the same number of poultry batches, from 13 slaughterhouses in Bulgaria. The samples were regularly collected and delivered during an 11-month period, from February until December, in 2008. Of the specimens, 172 came from northern Bulgaria and the rest came from southern Bulgaria. All of the samples were examined upon arrival to confirm their temperature and the condition of the individual sterile plastic bags with broiler carcasses. According to the recommendations, the temperature should be in the range of 2-8 °C; therefore, they were transported up to 24 h after chilling in cool boxes free of contamination and capable of maintaining the recommended parameters.

Every broiler carcass was removed from its plastic bag using sterile gloves and instruments. A piece of neck skin, 27 g in weight, was cut off and placed in a sterile blender bag (Kleinfeld Labortechnik, Germany). A volume (243 mL) of buffered peptone water (Merck, Germany) was added to the test sample and the initial suspension was mixed in a stomacher device (Stomacher®400 circulator, Seward Ltd., UK) for 1 min. A volume of 10 mL of the initial suspension was transferred to a glass container with 90 mL of Bolton broth (Merck).

Method of analysis

The detection and identification of *Campylobacter* spp. were performed according to the requirements of ISO 10272-1:2006, "Horizontal method for detection and enumeration of *Campylobacter* spp." (17).

In the enrichment step, we used liquid selective medium Bolton broth (Merck) as according to ISO 10272-1:2006. The microaerobic conditions during the incubation of the Bolton broth containers were achieved by leaving a free space of about 2 cm between the surface of the broth and the tightly fitted cap. The containers were incubated at 37 °C for 4 to 6 h and then at 41.5 °C for 40 to 44 h.

In the second stage of the isolation procedure, we used mCCD medium (Merck), and, as a second solid selective medium, Skirrow agar (Merck). The petri dishes were incubated at 41.5 °C in a microaerobic atmosphere for 48 h. The microaerobic conditions were achieved with sets of Anaerocult® C mini or an anaerobic jar (Merck) for the generation of an oxygen-depleted and CO_2-enriched atmosphere in the anaerobic jars. The nonselective medium Columbia agar (Merck), with 5% sterile sheep blood, was used to subculture the presumptive characteristic colonies from the 2 solid selective media.

Following the ISO 10272-1:2006 standard requirements, the presumptive characteristic colonies were examined for the morphology and motility of the microorganisms using the Gram and free drop techniques. The growth on the Columbia agar at 41.5 °C in aerobic conditions and at 25 °C in microaerobic conditions was also tested. To perform the biochemical confirmation and identification tests, we used oxidase disks, 30% hydrogen peroxide, indoxyl acetate substance, sodium hippurate, phosphate buffered saline, ninhydrin, and Mueller-Hinton agar with 5% sheep blood (all from Merck), as well as Brucella broth (HiMedia Laboratories Pvt. Ltd., India). Antimicrobial resistance was performed using nalidixic acid (30 μg) and cefalotin (30 μg)

disks (BioMérieux, France), the optical density of McFarland standard (BioMérieux), and a DEN-1 McFarland Densitometer (BIOSAN, Latvia).

Results

The analysis of our results, presented in the Table, indicates that 131 samples or 44.9% of all of the tested broiler carcasses were contaminated with *Campylobacter* spp. Two *Campylobacter* species, *C. jejuni* and *C. coli*, were primarily detected. In one of the specimens, a *C. lari* strain was also detected. The majority of the positive samples were found among the broiler carcasses from the slaughterhouses in northern Bulgaria, at 56.5%, while positive samples from the southern part of the country constituted 43.5%. *C. jejuni* strains were confirmed in 26 specimens (35.1%) from northern Bulgarian slaughterhouses; 1 isolate was confirmed as *C. lari* and the rest were *C. coli*. In the southern part of the country, the number of detected *C. jejuni* strains was 23, or 40.4% of all of the isolates. The remaining isolates, 59.6%, were identified as *C. coli*.

The data concerning the distribution of *Campylobacter* spp. in the broiler samples are presented in Figure 1. The predominant species in the positive samples was *C. coli*. About 61.8% of the isolates were identified as *C. coli* and 37.4% were *C. jejuni*. We also found 1 *C. lari*.

The results displayed in Figure 2 present the monthly distribution of *Campylobacter*-positive findings among all of the positive-tested broiler carcasses. June, July, and October 2008 were the months with the highest prevalence of *Campylobacter* isolates.

The data concerning the influence of the combination between the type of rearing and chilling method on *Campylobacter* spp. occurrence in broiler carcasses are presented in Figure 3. The highest

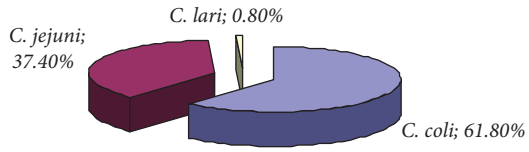


Figure 1. Distribution of *Campylobacter* spp. strains.

Table. Number of tested and positive samples in the slaughterhouses from the 2 parts of the country.

Slaughterhouses, part of the country	Type of chilling	Type of rearing	Tested samples	Positive samples	Percentage positive	*C. jejuni*	*C. coli*	*Other*
1/North	Spray	Conventional	20	11	55.0	3	8	
2/North	Spray	Free-range	41	11	26.8	5	6	
3/North	Immersion	Conventional	32	14	43.8	4	10	
4/North	Immersion	Free-range	15	3	20.0	1	2	
5/North	Spray	Conventional	21	9	42.9	4	4	1/*C. lari*
6/North	Immersion	Free-range	10	5	50.0	3	2	
7/North	Air	Conventional	12	6	50.0	2	4	
8/North	Spray	Conventional	9	4	44.4	1	3	
9/North	Spray	Conventional	12	11	91.6	3	8	
10/South	Air	Free-range	20	9	45.5	3	6	
11/South	Air	Free-range	70	31	44.2	16	15	
12/South	Immersion	Conventional	10	3	30.0	0	3	
13/South	Immersion	Conventional	20	14	70.0	4	10	
All			**292**	**131**	**44.9**	**49**	**81**	**1**

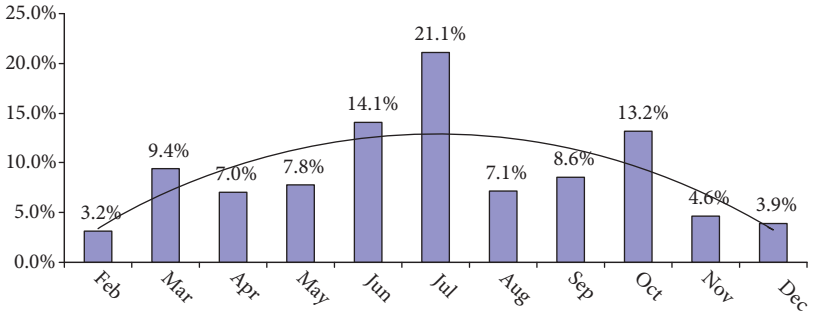
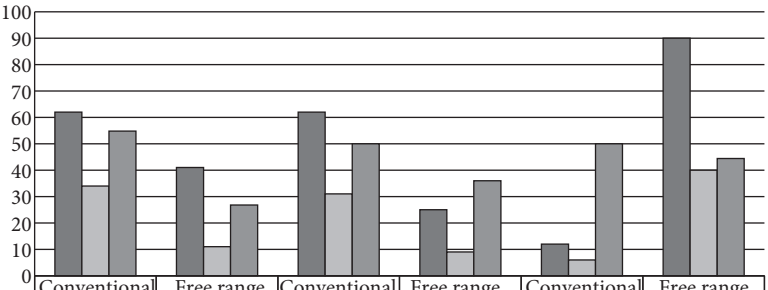


Figure 2. Month distribution of *Campylobacter*-positive samples.

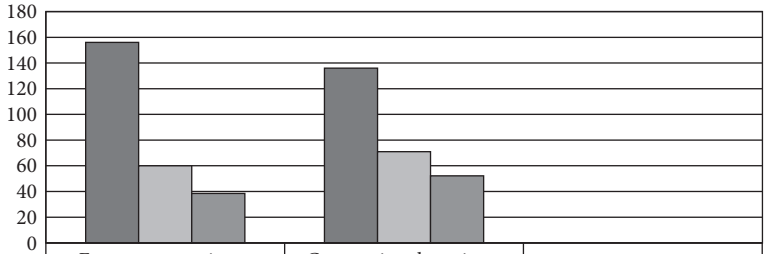


	Conventional spray	Free range spray	Conventional immersion	Free range immersion	Conventional air chilling	Free range air chilling
All samples	62	41	62	25	12	90
Positives	34	11	31	9	6	40
%	54.8	26.8	50	36	50	44.4

Figure 3. Influence of type chilling on presence of *Campylobacter* spp. on broiler carcasses.

percentage of positive samples was detected after the slaughter of conventionally reared broilers with spray chilling (54.8%), followed by immersion and air chilling (50.0%). In free-range reared broilers slaughtered and chilled using the spray technique, the lowest prevalence of *Campylobacter* spp. was 26.8%, followed by immersion at 36% and air chilling at 44.4%.

The relationship between the type of broiler rearing and the rate of contamination with *Campylobacter* spp. is presented in Figure 4. The carcasses of the broilers from farms with conventional rearing showed a higher percentage (52.2%) of *Campylobacter* compared to these from stocks reared on free-range farms (38.5%).



	Free range rearing	Conventional rearing	
All	156	136	
Positives	60	71	
%	38.5	52.2	

Figure 4. Type of rearing of examined broilers and contamination with of *Campylobacter* spp.

Discussion

According to EFSA reports, *C. jejuni* was the predominant species from the thermophilic *Campylobacter* spp. in poultry products in the majority of European countries (7-9). Our study clearly displays a prevalence of *C. coli*. EFSA reports concerning a 5-year period from 2003 to 2007 stated that the proportion of *Campylobacter*-positive broiler meat samples at slaughter varied from 0% to 86.5% within European Union member states. Many European countries reported high or very high levels (>20%) of positive samples. A few countries reported remarkably lower occurrences (0%-4.3%). The data submitted in 2007 revealed a large diversity between member states, from no positive samples in Romania to 55.8% and 86.5% positive samples in Spain and France, respectively (7). During 2008, the average *Campylobacter* prevalence in broiler carcasses at slaughter in the European Union was 75.8% according to data submitted by 26 member states, as well as Norway and Switzerland (8). A study in the United States revealed a prevalence of *Campylobacter* spp. in chilled broiler carcasses from 21.0% to 40.9% (18). Nachamkin (2) reported an isolation rate of *Campylobacter* spp. in chicken meat from 14% to 98%. In relation to that information, our results showed a relatively high prevalence of *Campylobacter* spp. at slaughter (44.9%), but this was significantly lower than the results obtained by Gavrila (19) in a study conducted in Romania, where the level of positive broiler carcass samples was very high: between 85% and 90.8%. In Bulgaria, a similar study, but involving a significantly lower number of samples, found 35.2% positive specimens among 35 frozen broiler carcasses and about 90% in chilled poultry (20).

In European countries, the predominant species isolated from fresh broiler meat was *C. jejuni*. The proportion of *C. jejuni* isolates ranged from 17.1% to 100% and the majority of European countries reported a level of isolation of more than 65% of all of the isolates. *C. coli* constituted less than 30% of the specified isolates, ranging, in fact, from 0% to 59% in most member states. In Norway and Slovenia, *C. lari* was found at a very low frequency (7). Other studies presented results confirming a marked prevalence of *C. jejuni* isolates from broiler slaughter carcasses and raw poultry meat over *C. coli* in the same products (21-23).

Figure 2 illustrates a clear seasonal variation of the positive findings among the tested broiler specimens. Our results are in correlation with the data reported by Nachamkin (2) concerning the seasonal variation of sporadic cases and outbreaks among humans, provoked by foodstuffs. Furthermore, they are similar to data from other European countries concerning mainly campylobacteriosis in humans, showing a peak of confirmed cases in the warmer months of the year (24). Other research has shown that the seasonal appearance of campylobacteriosis cases among the human population depended more on environmental factors than on the food sources (25). There is little officially published information concerning the *Campylobacter* spp. prevalence in Bulgaria and it mainly concerns the circulation of *Campylobacter* spp. pathogens in humans (26). The data reveal *C. jejuni* as the main pathogen inducing human gastroenteritis in Bulgaria. Our results confirm the world trend for poultry being a source of *Campylobacter* spp. infection.

The other aim of our study was to estimate the presence of *Campylobacter* spp. at the end of the slaughtering process. It is known that the key stages with great impact on the contamination of carcasses are scalding, evisceration, and giblet processing (27). A study in Bulgaria found that after scalding, contamination with *Campylobacter* spp. was low, but that evisceration led to a significant increase of up to 100%, and after the immersion chilling of the broiler carcasses, the contamination rate was about 72% (28). Comparing our data with respect to the type of chilling, we did not note significant differences between air, spray, and immersion chilling (45.1%, 43.7%, and 46.0%, respectively).

On the other hand, our results showed that the type of chicken flock rearing had a significant influence on *Campylobacter* prevalence. Conventional rearing resulted in a higher level of contamination of broiler carcasses with *Campylobacter* spp. (52.2%) compared to the free-range type (38.5%). Every examined broiler carcass was from a different batch, and our conclusion based on the data analyzed was that the conventional rearing system led to increased contamination rates with this pathogen. Nauta et al. (29) noted that monitoring of *Campylobacter* at the farm level for less than a week could result in false-

negative flocks, as once established, the prevalence of the pathogen increases dramatically within a week. Heuer et al. (30) concluded that the level of contamination depended on the type of production technology. The positive findings in flocks are generally higher (up to 100%) in organic and free-range flocks compared to intensively reared flocks, which is opposite of our findings. This presumably reflects the level of environmental exposure of such birds, as well as the older age of the birds at slaughter.

Our data showed that the isolates were mainly *C. coli* and *C. jejuni* strains. *C. coli* strains were the predominant species. We found a clear seasonal pattern of prevalence of *Campylobacter* spp. that corresponded with the data concerning human cases of foodborne campylobacteriosis during the warm months of the year worldwide. The free-range system of broiler rearing apparently had a beneficial effect on the rate of contamination of broiler carcasses. We also found that the type of chilling did not have a considerable impact on the rate of contamination of broiler carcasses with *Campylobacter*.

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