Prevalence of *Campylobacter* contamination in broiler meat

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SUMMARY

Food-borne campylobacteriosis is the most frequently reported zoonosis in the European Union (EU), which represents one of the leading public health issues and causes enormous financial losses (EFSA-ECDC, 2015.). Chicken meat is one of the most important global sources of the disease. Campylobacter contaminations of broiler flocks and chicken meat in Bosnia and Herzegovina (BiH) have been described. The aim of the study was to research the prevalence of campylobacter contamination of chicken carcasses and liver samples on slaughter line after the evisceration phase. Monthly sampling of chicken carcasses and liver was carried out along a year at one of the most recent broiler slaughterhouses in BiH (n=84). To isolate Campylobacter spp., from each carcass a deep sample of pectoral muscle, visceral cavity swab and liver (n=252) were taken. Campylobacter spp. contamination was detected in 27.4 % (23/84) of chicken carcasses. Out of the 252 analyzed samples, the most prevalent campylobacter contamination was observed in chicken breasts (19.0 %), less in visceral cavity (15.5 %), and the least in chicken liver (9.5 %). In total, Campylobacter spp. were isolated from 37 samples. C. jejuni was predominant (91.9 %), while C. coli was slightly represented (8.1 %). Results of the study underline the importance of chicken meat as a potential source of food-borne campylobacteriosis and suggest compulsory microbiological control of campylobacter contamination of chicken meat in BiH.

Key words: *Campylobacter spp., C. jejuni, C. coli, chicken meat, Bosnia and Herzegovina*

INTRODUCTION

Food-borne infections and intoxications remain one of the most important public health issues globally, both in developing and in developed countries. Numbers of reported outbreaks of food-borne diseases and diseased people are constantly growing. For instance, a total of 320,000 of cases of food-borne diseases, caused by only 6 most prevalent bacterial pathogens (non-typhoidal *Salmonella* spp., *Campylobacter* spp., *Listeria monocytogenes*, *Escherichia coli*, *Yersinia enterocolitica* and *Brucella melitensis*) was reported in the EU in 2011, but it has been estimated that the actual number of cases is probably much higher, and the reasons for such extent of the problem are numerous (EFSA-ECDC, 2013).

Many agents contaminate food, originating from people and/or animals or from the environment during the process of food production, processing, distribution, storage or consumption. Due to inadequate manipulation with raw materials, semi-products and final products, and under favorable conditions in foods or digestive tract, the pathogens may multiply to critical levels (usually ≥ 106/g or mL), adequate to cause infection or produce toxins a quantity of toxin in quantities sufficient to provoke clinical manifestation of food-borne intoxica-

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tion. For example, human infective dose of *C. jejuni* may be less than 500 cells/g or mL of food (Robinson, 1981.).

According to report of the European Food Safety Authority (EFSA), human food-borne campylobacteriosis is the most prevalent reported zoonosis in EU, with 236,851 confirmed cases in 2014, and with a 10 % increase in number of cases comparatively to 2013 (EFSA-ECDC, 2015.). Economic consequences of *campylobacter* infections are significant; it was estimated that the overall EU financial loss due to *campylobacter*iosis total to approximately 2.4 billion of euros (EFSA-ECDC, 2013).

Campylobacter species were isolated from various foods, among which chicken meat is dominant, and *campylobacter* contamination of chicken meat broadly varies worldwide. Suzuki and Yamamoto (2009.) describe a wide range of prevalence of *Campylobacter* spp. contamination of chicken meat, and illustrate that the prevalence was estimated at 8.1 % in Estonia, 17 % in Belgium, 19.1 % in average in countries of former Soviet Union (Ukraine, Belarus and Moldova), 25.1 % in Switzerland, 45.6 % in Germany, 58.8 % in France, 80 % in Italy, 89.1 % in New Zealand, 90.4 % in Oceania, and 100 % in Australia.

Prevalence of campylobacter contamination of retail meat chicken in BiH was estimated in the range from 34.7 % (Uzunović-Kamberović et al., 2007) to 45.5 % (Uzunović and Smole Možina, 2013.). Also, prevalence of *Campylobacter* spp. contamination in BiH was described for fecal samples from broiler flocks (62.0 %), and for carcass and skin samples of broilers on slaughter line (58.1 %) (Hadžiabdić et al., 2013). It is well known that *Campylobacter* spp. contamination of chicken meat poultry in slaughterhouse is due to the meat cross-contamination along various technological phases of slaughtering and processing, such as evisceration of the carcasses (Izat et al., 1988; Mead et al., 1995; Guerin et al., 2010). Having in mind the proven role of chicken meat as one of the most important sources of food-borne campylobacteriosis, and the described campylobacter-contamination of the broiler flocks and chicken meat from slaughter line and from retail in BiH, the goal of our research was to investigate the prevalence and intensity of campylobacter contamination of broiler carcasses and liver sampled after the evisceration and before chilling in a modern broiler slaughterhouse in BiH.

MATERIALS AND METHODS

Research was carried out in one of the largest broiler slaughterhouses in BIH. Production process in the slaughterhouse is certified according to the ISO 9001 and the ISO 14001 standards, and in line with requirements of HACCP food safety and Halal food quality management systems as well. Whole production process is under permanent control by relevant veterinary inspection.

Samples of broiler carcasses were taken from the slaughter line after the evisceration phase, prior to the chilling phase. Monthly random sampling was done for one-year period. Each month seven random samples of broiler carcasses and their matching livers were aseptically taken off the production line. To account for variability of the production process, each sampling was performed on different working days and hours. The carcasses and their livers were separately packed in sterile plastic bags and immediately transported to the laboratory in portable refrigerators under controlled temperature of 2-5°C. In total, 84 carcasses and 84 matching livers were sampled along one-year period. In the laboratory, from each carcass was taken one deep sample (25 g) of the pectoral muscle (m. pectoralis) and one swab of visceral cavity, which, as we well as the matching liver, were subjected to further bacteriological isolation and identification of *Campylobacter* spp. (n=252). A carcass was considered as campylobacter-positive if any of its samples of m. pectoralis, visceral cavity swab, or liver showed contamination with *Campylobacter* spp.

The international standard method ISO 10272 (ISO, 1995) was followed for the isolation and identification of *Campylobacter* spp. Initial suspension of the samples were enriched in 45 mL of selective Preston broth (4012862, 4240017, Biolife) with 5 % sterile lyzed horse blood and incubated for 18 hours at 42°C in microaerophilic atmosphere (CampyGen CN0025, Oxoid). One loopful of the broth was parallely streaked on Karmali agar (4012832, 4240035, Biolife) and Skirrow agar (M144, FD008, Himedia and 5 % of lyzed sheep blood), which were incubated for 5 days at 42°C in microaerophilic atmosphere. From each Petri plate up to 5 presumptive colonies were subjected to identification. Identification of *Campylobacter* spp. isolates was based on morphological characteristics of the colonies and bacterial cells, biochemical properties and motility, and their resistance to nalidixic acid and cephalotin (ISO, 1995). Brucella broth (4012742, Biolife) was also used for incubation at 25°C and 42°C in microaerophilic atmosphere up to 5 days, where thermotolerant campylobacter species displayed growth at 42°C, but not at 25°C. To test antimicrobial resistance of the isolates, Mueller-Hinton agar II (4017402, Biolife), cephalotin and nalidixic acid disks (both 30 µg, Liofilchem) were used, with incubation for 24 hours at 37°C in microaerophilic atmosphere.

RESULTS AND DISCUSSION

Our results confirm *Campylobacter* spp. contamination of chicken meat in the phase of carcass evisceration. The total of 84 chicken carcasses and their matching livers was sampled, out of which in 23 (27.4 %) *Campylobacter*

Table1 Distribution of *Campylobacter* spp. isolates per site (sample) of isolation and identified species in campylobacter-positive broiler carcasses (n=23).

Carcass	No. of *Campylobacter* spp. isolates	No. of isolates		Origin of the isolates		
		C. jejuni	*C. coli*	*m. pectoralis*	swab[1]	liver
1	1	1	-	+	-	-
2	1	1	-	+	-	-
3	2	2	-	+	+	-
4	1	1	-	-	+	-
5	2	2	-	+	-	+
6	1	-	1	-	C.coli	-
7	1	1	-	-	+	-
8	1	1	-	-	-	+
9	1	1	-	-	+	-
10	3	2	1	C.coli	+	+
11	2	2	-	+	+	-
12	3	3	-	+	+	+
13	3	2	1	+	C.coli	+
14	1	1	-	+	-	-
15	3	3	-	+	+	+
16	3	3	-	+	+	+
17	2	2	-	+	+	-
18	1	1	-	+	-	-
19	1	1	-	-	-	+
20	1	1	-	+	-	-
21	1	1	-	+	-	-
22	1	1	-	-	+	-
23	1	1	-	+	-	-
Total	**37**	**34**	**3**	**16**	**13**	**8**

[1]:swab of the visceral cavity; +: *C. jejuni*

spp. was confirmed (Table 1). Previous studies state wide variations of the slaughterhouse prevalence of *Campylobacter* spp. contamination of chicken meat after the evisceration. Hartog et al. (1983) calculated a 27.5 % campylobacter contamination of chicken meat after evisceration, which is almost identical to our finding (27.4 %). In addition, our finding is very similar to those of a South African research (Bartkowiak-Higgo et al., 2006), which confirmed campylobacter contamination after the evisceration in 12 of 50 (24.0 %) chicken carcasses. Lower prevalence (6.3 %) was reported by Karolyi et al. (2003), while investigating the contamination after evisceration in the production line with different chilling systems. On the other side, much higher values of prevalence of campylobacter contamination after the evisceration phase were reported, from 75.0 % (Adesiyun et al.,1992) up to even absolute 100 % contamination (Rosenquist et al., 2006). Interestingly, our results differ from those of Hadžiabdić et al. (2013) who calculated a 58.1 % *campylobacter* contamination of broiler carcasses and skin in slaughter line in BiH. Nevertheless, in contrary to our research, these authors analyzed only the carcasses of broilers from campylobacter-positive flocks.

Contamination with *C. jejuni* only was confirmed in 20 of the 23 (87.0 %) campylobacter-contaminated broiler carcasses, while coexistence of *C. jejuni* and *C. coli* was observed in two carcasses (8.7 %). Only one carcass (4.3 %) exhibited sole contamination with *C. coli* (Table 1). In total, *Campylobacter* spp. were isolated from 37 samples (Table 3), among which an absolute predomination of *C. jejuni* (34/37; 91.9 %) was observed, while *C. coli* was identified in only 3 isolates (8.1 %) (Graph 1). Our finding of *C. jejuni* domination over *C. coli* agrees with other studies that establish similar relation between the two thermoresistant *campylobacter* species in various types of chicken meat samples (FDA, 2010; Luu et al., 2006.; Williams and Oyarzabal, 2012.; Kovačić et al., 2013.; Hadžiabdić et al., 2013).

In the previous research on campylobacter contamination of broiler flocks and their carcasses on slaughter line in BiH (Hadžiabdić et al., 2013), a clear dominance of *C. jejuni* (79.55 %) over *C. coli* (20.45 %) among 44 *Campylobacter* spp. isolates was described. Other authors also describe such domination of C.jejuni over C.coli in chicken meat samples taken in slaughterhouses. After research on prevalence of *Campylobacter* spp. in chicken meat from slaughterhouse and retail, Denis et al. (2001) stated that 44.8 % of 49 the slaughterhouse samples showed campylobacter contamination, out of which 87.5 % were contaminated with *C. jejuni* and 12.5 % with *C. coli*, which argues in favor of our finding on proportion of the species. In contrast to our finding, Uzunović-Kamberović et al. (2007) observed higher prevalence of *C. coli* (56.9 %) than of *C. jejuni* (41.2 %) contamination of various samples of chicken meat from retail in Zenica-Doboj Canton (BiH). Such difference in the results may be due the difference in sampled populations, since Uzunović-Kamberović et al. (2007) collected 147 samples of fresh and frozen chicken meat (25 of liver and 122 of leg skin) from 53 different markets in Zenica-Doboj Canton, where the samples originated from 14 national and 7 foreign producers.

Intensity and extent of campylobacter contamination in chicken carcasses are shown in Table 2. The highest intensity of the contamination, i.e. presence of *Campylobacter* spp. in all of the three samples (liver, swab of visceral cavity and m. pectoralis) was detected in 5 of 23 campylobacter-positive carcasses (21.7 %). Contamination of one out of the three samples was confirmed in 14 *campylobacter*-positive carcasses (60.9 %); among which the contamination of m. pectoralis was observed in 7 of the 14 carcasses, 5 carcasses showed only contamination of the visceral cavity, while 2 carcasses displayed sole contamination of the liver. Contamination of two out of the three samples was observed in the rest of 4 campylobacter-positive carcasses (17.4 %), where m. pectoralis was contaminated in all of the four carcasses, and only one carcass showed *campylobacter* contami-

Table 2 Intensity and extent of *Campylobacter* spp. contamination of broiler carcasses (n=84).

Intensity of carcass contamination	n	% positive	No. of contaminated samples		
			m. pectoralis	swab[1]	liver
One sample positive	14	60,9	7	5	2
Two samples positive	4	17,4	4	3	1
Three samples positive	5	21,7	5	5	5
Total	23	100,0 %	16	13	8

[1]: swab of the visceral cavity.

Table 3 Distribution of *Campylobacter* spp. contamination of broiler carcasses per site (sample) of isolation and identified species.

Sample	n	*Campylobacter* spp.		*C. jejuni*		*C. coli*	
		No. positive	% positive	No. positive	% positive	No. positive	% positive
M.pectoralis	84	16	19,0	15	17,9	1	1,2
Liver	84	8	9,5	8	9,5	-	-
Swab[1]	84	13	15,5	11	13,1	2	2,4
Total	252	37	14,7	34	13,5	3	1,2

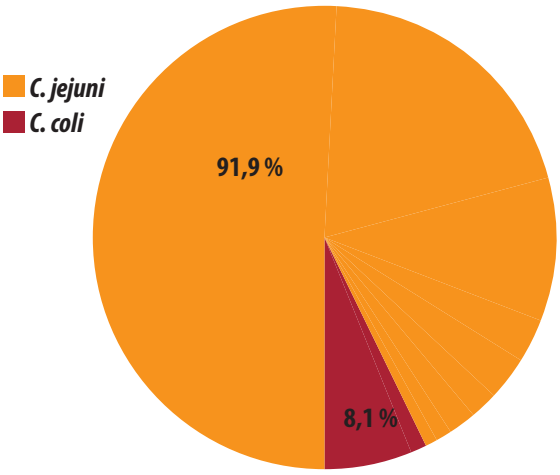
[1]: swab of the visceral cavity.

nation of the liver solely. These results imply that the pectoral muscle tissue was the most frequent predilective locus of the campylobacter carcass contamination in our research, while the liver was the least frequently contaminated with *Campylobacter* spp. In the investigation carried out at a chicken slaughterhouse in northern Germany, Stoyanchev (2004) evidences campylobacter contamination of chicken breasts (16.6 %) and liver (53.3 %), which is in contrast to our findings of the spread of *campylobacter* contamination in the carcasses. On the other side, similar to our results. The most prevalent species was *C. jejuni* (65.2 %), while *C. coli* was much less prevalent (30.4 %). The reason for differences in intensity and spread of the contamination of chicken breasts and liver between our and the German study may be in that Stoyanchev (2004) based the results on 30 carcasses from 3 broiler flocks only, without stating the season of the sampling, i.e. not taking into account previously described seasonal variations of the pathogen in Germany (Atanassova and Ring, 1999). It has been known for a long time that there are significant seasonal differences in prevalence of campylobacter contamination of chicken meat (Willis and Murray, 1997).

A sample from deep of the breasts (m. pectoralis), a swab of the visceral cavity, and the matching liver was bacteriologically tested in the laboratory, which totaled in 252 samples (Table 3). *Campylobacter* spp. contamination was confirmed in 37 of the 252 analyzed samples (14.7 %). *C. jejuni* was identified in 34 (13.5 %), a *C. coli* only in three samples (1.2 %).

Microbiological analysis of the 84 liver specimens showed that eight (9.5 %) of the samples were positive for campylobacter contamination. *C. jejuni* was isolated from all of them, i.e. none of the positive samples showed contamination with C. coli. On the other hand, campylobacter contamination of visceral cavity was confirmed in 13 (15.5 %) carcasses, where *C. jejuni* was identified in 11 (13,1 %), and C.coli in two (2,4 %) carcasses. Our estimates are lower than results of Ivanović (2001), who calculated a 40.0 % prevalence of *C. jejuni* contamination of chicken liver, and a 6.1 % contamination with *C. coli*, while the prevalence of *C. jejuni* contamination of visceral cavity was estimated at 58.33 %. However, the Ivanović's research was carried out in free-range chickens slaughtered in households, without any control, which probably resulted in higher prevalence estimates. Besides that, prevalence estimates of campylobacter contamination of chicken liver may vary in a wide range, from 28.6 % (Denis et al., 2001) to even 92.9 % (Fernandez and Pison, 1996). It is also known that prevalence of *Campylobacter* spp. in swabs of chicken carcasses and visceral cavity may range widely. Accordingly, *campylobacter* species were detected in none of 75 swabs of chicken carcasses (Granić et al., (2009.), while Berndtson et al., (1992) stated *Campylobacter* spp. contamination of 93 % of visceral swabs of the visceral cavity.

Sixteen of 84 samples of m. pectoralis showed *Campylobacter* spp. contamination (19.0 %), where 17.9 % of the samples were contaminated with *C. jejuni*, while *C.coli* contamination was confirmed in only one sample (1.2 %). Our results of C.jejuni contamination are near to those of Kovačić et al., (2013), who detected the contamination in 14.6 % of 547 samples of chicken meat from Dalmatia, Croatia. On the other side, Kazuaki and Katsuhiko (1999) confirmed the prevalence of *C. jejuni* contamination of chicken meat produced in Japan at 45.8 %, but also estimated the prevalence for imported chicken meat at 3.7 %, which argues in favor of wide variation of prevalence of *campylobacter* contamination of chicken breasts. Accordingly, Gritti et al. (2011) investigated prevalence of *Campylobacter* spp. in 24 samples of chicken breasts and tighs in Brazil and did not isolate the pathogens from any of the samples. Contrarily, similar investigation conducted in Malaysia (Tang et al., 2009) estimated a 95 % *Campylobacter* spp. contamination in 22 samples of chicken breasts. Williams and Oyarzabal (2012) declared a 41 % prevalence of *Campylobacter* spp. in 755 samples of chicken meat, where *C. jejuni* was found in 66 % and *C. coli* in 28 % of the samples. In a Vietnamese research (Luu i sur., 2006.), *Campylobacter* spp. contamination of fresh chicken breasts was confirmed in 31 of 100 tested samples, where C.jejuni was present in 45.2 %, and C.coli in 25.8 % of the samples. In the annual report on antimicrobial resistance of bacterial pathogens isolated from



Graph1. **Proportions of *C. jejuni* and *C. coli* identified among *Campylobacter* spp. isolates (n=37).**

retail meat in 11 states of the USA (California, Colorado, Connecticut, Georgia, Maryland, Minnesota, New Mexico, New York, Oregon, Tennessee and Pennsylvania) during the 2002-2010 period, the prevalence of *Campylobacter* spp. in chicken breasts was estimated at more than 90 %, where an approximate ratio of prevalence of *C. jejuni* vs. C.coli was 2:1 (FDA, 2010.). The differences in prevalence of *Campylobacter* spp. contamination of the samples analyzed in our research and the findings of other authors may be explained by the facta that the carcasses in our research were relatively fast transported from the slaughterhouse and analyzed in the laboratory, which did not left sufficient time for the pathogens from the carcass surface to penetrate to the deeper tissues. Finally, having in mind the described food safety and quality in place an the slaughterhouse, a lower overall campylobacter contamination of the production process may be assumed, which certainly may be subject of future research.

CONCLUSIONS

Our results confirm *Campylobacter* spp. contamination of chicken carcasses in slaughterhouse industry in BiH, which is in agreements with findings of other investigations of slaughterhouse and retail samples in BiH. The observed *campylobacter* contamination was not highly intensive, because the most prevalent were the carcasses with only one contaminated sample, while less frequent were the carcasses that displayed two or three contamination sites. The highest prevalence of campylobacter contamination was evidenced in chicken breasts; less frequent was the contamination in the visceral cavity, while the least prevalence was detected in chicken livers. An absolute domination among *Campylobacter* spp. isolates showed *C. jejuni*, while *C. coli* was slightly represented. In most of the *campylobacter*-positive carcasses exclusive *C. jejuni* contamination was detected, sole *C. coli* contamination was observed in one carcass, while two carcasses displayed mixed contamination with both of the pathogens. Results of our research surely underline the necessity of inclusion of *Campylobacter* spp. in monitoring the microbiological safety of food produced in poultry industry in BiH, and may initiate further broader research on the issue of campylobacter contamination of food.

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AUTHOR INSTRUCTIONS

In the Meso journal all categories of scientific papers, expert papers, authors' reviews, presentations from scientific and expert conferences as well as other thematically acceptable articles in Croatian and English are published.
The papers are subject to review.

Content and volume of articles

The headline of the article should be concise. The names of the authors should follow the title. Titles and addresses should be indicated on a separate sheet of paper. Every author should provide: academic degree, name and address of the organisation in which is employed, so as function in the organisation in which is employed.

For easier contact authors needs to provide telephone number, fax and email address. Telephone and fax numbers will not be published in the journal.

Every discussion must have a short summary in Croatian and English. Below the summary three to five key words must be stated.

The names of those authors that are quoted in the text and the year of publishing must be stated (in brackets). If more than three authors wrote the quoted article, the surname of the first one is mentioned, and add et all., followed by the year of publishing. A list of References should be arranged alfabetically, as follows:

a) Article in the journal:
Abu-Ruwaida, A. S., W. N. Sawaya, B. H. Dashti, M. Murard, H. A. Al-Othman (1994): Microbiological quality of broilers during processing in a modern commercial slaughterhouse in Kuwait. J. Food Protect. 57, 887-892.

b) Proceedings:
Guerra, M., F. Bernardo (1997): Occurrence of Listeria spp. in traditional cheeses from Alentejo, Portugal. World Congress of Food Hygiene. The Hague, The Netherlands, 1997 August 24-29. Proceedings, p.214.

c)Book of abstracts:
Hadžiosmanović, M., L. Kozačinski, Ž. Cvrtila (2002): Shelf life of fresh poultry meat. Technology - food - nutrition - health, CEFOOD Congress, Ljubljana, September 22-25, 2002. Book of Abstracts, p. 99.

d) Book:
Gracey, J., D. S. Collins, R. J. Huey (1999): Meat hygiene. Tenth edition. W. B. Saunders company Ltd London, Edinburg, New York, Philadelphia, Sydney, Toronto.

The original (up to 15 typed pages) should have all the pictures, drawings, and diagrams. Supplements (charts, diagrams and pictures) are enclosed separately, at the end of the work. All appendices, graphs, photos and pictures must be bilingual (Croatian and English). Charts and photographs should be delivered in one of the graphic or image formats (.xls, *.tif or *.jpg)*

It is recommended to write in Word (Microsoft) programme, to use Word (Microsoft) or Excel (Microsoft) for charts.

Article with all supplements should be sent to one of the following emails:
meso@meso.hr / klidija@v ef.hr / zcvrtila@vef.hr