OCCURRENCE OF *CAMPYLOBACTER* SPP. IN ROMANIAN BROILER CHICKEN PRODUCTION SECTOR

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Abstract

Due to the well-known potential of Campylobacter spp. to determine illness in humans, its detection and occurrence, especially in poultry meat (due to the frequent contamination of this food product) are considered highly important for the consumer's health point of view.
In order to determine the occurrence of Campylobacter spp. in the Romanian broiler chicken production sector, a number of five units were selected and samples were collected as it follows: a total number of 600 samples consisting of chicken skin neck, 600 samples of intact intestines and 1200 samples of fresh chicken carcasses.
The results were different from one unit to another, but overall, 54.7 % of the caecal material samples, 48.5 % of the neck skin samples and 31,5 % of the carcass samples were contaminated with Campylobacter spp., with an overall percentage of positive samples reaching 60,2 %. This high occurrence opens the opportunity for future research in order to determine the causes leading to contamination, while also identifying the species of this genus, for a better understanding of this mechanism through which this foodborne pathogen contaminates broiler meat.

***Keywords*:** broiler *carcass, Campylobacter, food safety, slaughterhouse.*

INTRODUCTION

Campylobacter spp. are well known to be able to determine illness in humans, especially the thermotolerant strains, such as *Campylobacter jejuni* and *C. coli*, the most commonly reported bacterial causes of human infections in the European Union (EU) (Hermans et al., 2011; Habib I. et al., 2012; Kovalenko et al., 2013). In 2010, the campylobacteriosis cases per 100,000 EU inhabitans were confirmed to reach 48.6 % of the total (EFSA, 2012). Fica et al. (2011) mentions gastroenteritis cases produced through *Campylobacter* infection and the possibility that this pathogen may also determine several complications, such as Miller-Fisher and Guillain-Barré syndromes.

C. jejuni and *C. coli* are Gram-negative rods, with a characteristic motility, but compared to several other foodborne pathogens, more fragile and requiring microaerobiosis for multiplication (Park, 2002; Rodgers et al., 2012).
It is well known that this foodborne pathogen is a frequent factor of contamination for poultry meat (Friedman et al., 2004; Malher et al., 2011), in 2010 the frequency of *Campylobacter*-contaminate broiler meat samples varied from one Member State to another, with ranges of 3.1 % and 90 % (EFSA, 2012). This is usually caused by the poor or insufficient biosecurity in and around the poultry farm (Newell and Fearnley, 2003; Thakur et al., 2012). Van Gerwe et al. (2005) showed that in a flock comprising 20,000 broilers, the prevalence of *Campylobacter* can increase from 5 % to 95 % in only six days after the inoculation. Also, in the slaughterhouse, the chances of cross-contamination are also increased. Contamination has been proven to happen in steps such as scalding, evisceration and water chilling (Hue et al., 2010; Jacobs-Reitsma, 2000).
The aim of this presence is to determine the occurrence of *Campylobacter* spp. in broiler chicken production at slaughterhouse and retail level in Romania, for 2011.

MATERIALS AND METHODS

Sampling
A total number of 600 broiler chicken neck skins, 600 number of broiler chicken intact intestine an 1200 fresh broiler chicken carcasses were collected from five different units, during the year 2011. All the samples were collected monthly, 10 samples monthly from each unit, consisting of broiler chicken neck skins, another 10 samples of intact intestines and 20 samples of fresh broiler chicken carcasses. The intact intestines samples were taken during evisceration step, and placed all together in a single sterile plastic bag. Neck skin samples were collected separately and placed each in sterile plastic bags. During the same day, carcass samples were collected, but not from the same slaughter batch as the other two categories of samples. All chicken carcasses produced in the five selected units included in this study were usually sold in tight, sealed plastic bags. The samples were introduced in a cooler, in order to be kept at a temperature of 4-6^{o}C and transported to the laboratory for microbiology analyses.

Isolation and identification of Campylobacter spp.

A quantity of 10 g of chicken back skin from the carcass and another 10 g of neck skin were aseptically taken and introduced in sterile bags, for the enrichment step. Afterwards, the sterile bags were filled with 90 mL sterile Bolton Broth and introduced in a stomacher for one minute. Further on, they were incubated under microaerobic conditions at 37^{o}C, for 46 h, followed by 41.5^{o}C for 44 h (Kovalenco et al., 2013). After enrichment, 10 µl of the enriched broth was plated on mCCDA agar and incubated for 48 h at 42^{o}C under microaerobic conditions. From the colonies on mCCDA agar, several typical ones were streaked on Columbia blood agar, the plates being further on incubated for 24 h at 41.5^{o}C in microaerobic conditions.

Concerning the intestines, the caeca was selected for the identification. Caecal material from the 10 samples of intestines was analyzed separately, 1 g of content being selected for each, for further analysis.

For the identification, according to ISO 10272-1:2006, bacteria isolated from broiler chicken material that showed typical growth on mCCDA, were Gram-negative, with specific corkscrew motility, oxidase positive and without any growth at 41.5^{o} C in aerobic conditions and growth at 25^{o}C in microaerobic conditions, were considered as *Campylobacter* spp.

RESULTS AND DISCUSSIONS

The results showed a high *Campylobacter* colonization for caecal samples and a high contamination of neck skin samples in the five units chosen for this study (Table 1).

The present results showed that between the chosen units, there are differences concerning the contamination for the different collected samples. Concerning the samples of caecal content, the highest contamination percentage was shown for unit D, while the lowest for unit A. Overall, from the total number of analyzed samples, 328 showed positive results. Concerning the neck skin samples, overall 48.5 % of the total numbers of samples were shown to be contaminated with *Campylobacter* spp, the highest number of positive results among the analyzed ones pertaining to unit C. For the carcass samples, the highest number of samples with positive results was shown in the samples collected from unit D, while the lowest among the ones collected in unit A. Overall, 31.5 % of the total number of samples were contaminated with *Campylobacter* spp. (Figure 1).

Table 1. The percentage of *Campylobacter* spp. positive broiler chicken samples in five Romanian units in 2011

Unit	*Campylobacter* spp. positive samples percentage (no. positive/total no.)			
	Separate caecal material	**Neck skin samples**	**Carcass samples**	**Total number**
A	48,3 (58/120)	45,8 (55/120)	59,2 (142/240)	53,1 (255/480)
B	53,3(64/120)	40,0 (48/120)	72,9 (175/240)	59,8 (287/480)
C	49,2 (59/120)	55,8 (67/120)	67,5 (162/240)	60,0 (288/480)
D	62,5 (75/120)	51,7 (62/120)	78,3 (188/240)	67,7 (325/480)
E	60,0 (72/120)	49,2 (59/120)	66,3 (159/240)	60,4 (290/480)
All	54,7 (328/600)	48,5 (291/600)	31,5 (757/1200)	60,2 (1445/2400)

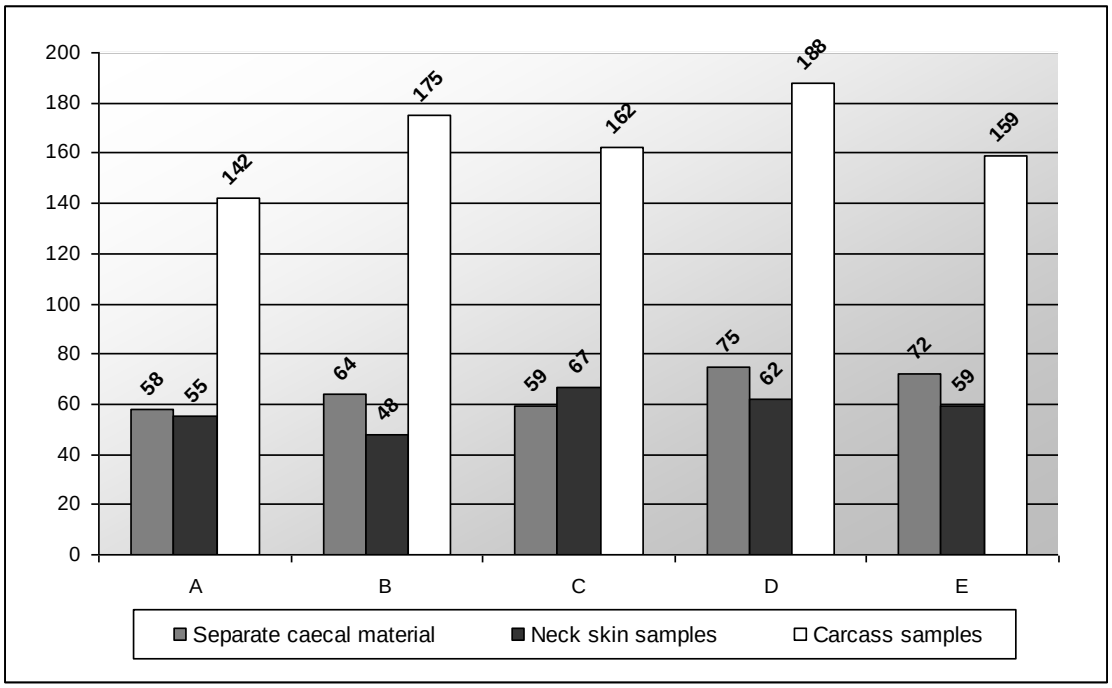

Fig. 1. The number of positive results for *Campylobacter* spp. identification, for separate caecal material, neck skin samples and carcass samples in each unit included in the study

For the monthly evolution for the entire year of analysis, data are presented in figure 2. During the months of 2011, the total number of positive results for the carcass samples as well as for the caecal material showed several variations, with the highest number for April and August, while the lowest were observed for January.

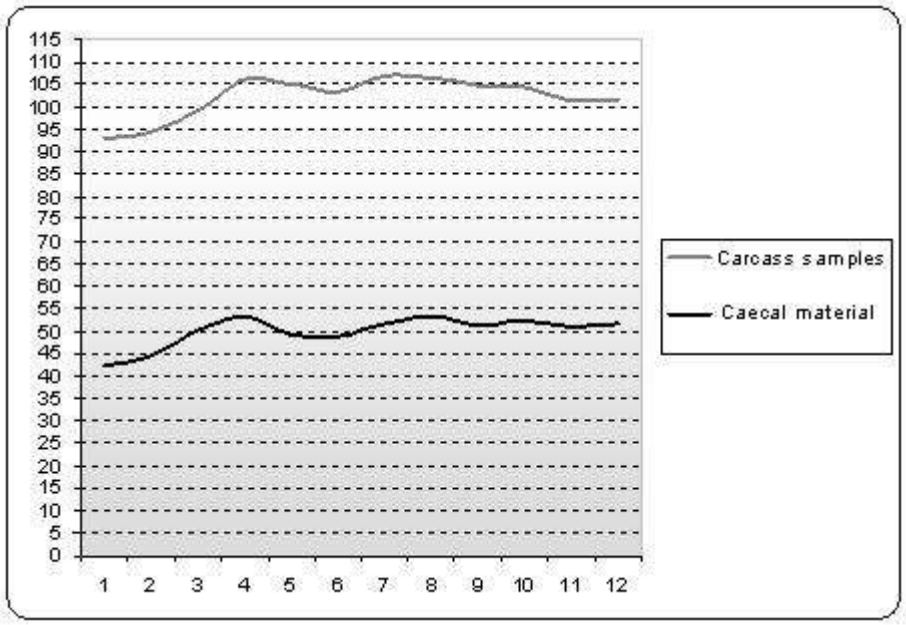

Fig. 2. The monthly percentage of positive samples for the presence of *Campylobacter* spp. – caecal material and carcass samples analysis

CONCLUSIONS

In conclusion, this study revealed a high *Campylobacter* spp. contamination in the slaughterhouses and also on retail level. There is a need for a state *Campylobacter* monitoring and control programs. Further on, research could show the causes for this high contamination, as well as the mechanisms needed for *Campylobacter* spp. to survive during the processing.

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