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INCIDENCE AND MICROBIAL DIVERSITY OF *CAMPYLOBACTER* SPP. ISOLATES DURING THE SLAUGHTERHOUSE PROCESSING OF POULTRY AND CRITICAL CONTROL POINTS OF THE PROCESS

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Summary

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The microbial contamination with *Campylobacter* spp. was investigated throughout the entire technology of slaughterhouse processing of five herds of broiler chickens. The organism was isolated in 76.2% of studied samples. *Campylobacter* spp. were present at all stages of the process. Immediately after scalding, the contamination with *Campylobacter* spp. was the lowest (20%) whereas after evisceration it reached 100%. The contamination of poultry carcasses following the water chilling was 72%. The most frequently isolated *Campylobacter* organism was *C. jejuni* (80.9%), followed by *C. coli* (19.1%). The further differentiation of *C. jejuni* into subspecies showed that *C. jejuni subsp. jejuni* was isolated in 69.3% of the specimens while in 30.7% of cases, *C. jejuni subsp. doylei* was detected.

Our data showed that the defeathering, the evisceration and the water chilling were the critical control points of cross contamination of birds in a poultry slaugtherhouse.

Key words: *Campylobacter coli*, *C. jejuni*, immersion chilling, poultry, slaughterhouse processing

INTRODUCTION

Campylobacter infections are foodborne diseases, caused mainly by poultry and poultry products (Stern *et al.*, 1995; Alterruse *et al.*, 1999; Corry and Atabay, 2001). Recently, the cases of campylobacterial gastroenteritis in men in some western industrial countries occurred more frequently than those, caused by salmonellae (Rautelin and Hanninen, 2000; Wedderkopp *et al.*, 2000). The problem becomes more and more important with the implementation of contemporary requirements of foodstuffs safety.

In the "from stable to table" chain, the poultry slaugherhouse is a principal source of bacterial contamination of poultry meat (Bryan and Doyle, 1995). A considerable part of live broiler chicken batches that are received in abattoirs, are carrying *Campylobacter* in their gut as well as on their feathers and skin (Jacobs-Reitsma *et al.*, 1994; Mead *et al.*, 1995). This is a prerequisite for an extensive dis-

tribution of the microbial pathogen during the slaughtering. The different technological stages are responsible for either decrease or increase of microbial contamination (Bryan and Dayle, 1995). The data about the prevalence of *Campylobacter* throughout the whole of the operation of poultry carcasses processing are yet incomplete and variable.

Several authors consider that the places at high risk for occurrence of bacterial cross contamination with *Campylobacter* are the scalding tank, the plucker, the evisceration trough and the chilling tank (Bryan and Doyle, 1995; Anonymous, 1997; Ono and Yamamoto, 1999). The extraction of feathers from the follicles permits a deep penetration of *Campylobacter* organisms that could hardly or not at all be removed during the subsequent stages of the processing (Mead *et al.*, 1995). During the evisceration, the transfer of microorganisms continues from the poultry carcass to the equipment, the hands of personnel and the surfaces that are in contact with the carcass and then, toward other carcasses (Bryan and Doyle, 1995). According to Padungtod *et al.* (2002), during the final washing and chilling, there is a trend toward reduction of microbial counts, including *Campylobacter* but not toward their complete elimination. Following immersion chilling, 51% of specimens obtained from the skin surface, were positive for *Campylobacter* (Jones *et al.*, 1991).

The present study aimed to determined the degree of the contamination with *Campylobacter* of broiler chickens, received in the slaugherhouse, to determine the microbial diversity of *Campylobacter* spp. isolates throughout the processing and to specify the critical control points of the poultry processing technology.

MATERIALS AND METHODS

The present study for *Campylobacter* incidence was performed on 185 specimens obtained from 5 batches (herds) of broiler chickens, each consisting of 1500 birds, at various technological stages of poultry processing in a slaughterhouse located in Central Bulgaria. The broiler chickens originated from poultry farms situated in different regions of the country – Bourgas, Haskovo, Stara Zagora. Specimens were obtained via swabbing the skin surface of birds at the following technological stages: bleeding, scalding (at 58 ^{o}C), defeathering, evisceration and chilling. At each of those stages, 5 specimens from previously specified birds from each batch (a total of 125 specimens) have been obtained.

The specimens were collected in the chest region from an area of approximately 20 cm^2 via sterile cotton swabs, dampened in sterile saline solution. The swabs were then put into enrichment broth.

During evisceration, one of the caeca of each specified bird was collected (n=25). Under laboratory conditions, part of its content was inoculated in enrichment broth and on selective *Campylobacter* agar.

Specimens from the personnel, performing the manual removing of poultry guts after the evisceration were obtained via swabbing the skin surface of the hands (n=25). Also, 1 mL specimens of water from the beginning (n=5) and the end (n=5) of chilling tank were collected and added to 9 mL enrichment broth.

The cultivation of specimens was done in an enrichment thioglycolate broth (Merck, 1.08190) and on selective *Campylobacter* agar (Merck, 1.02248), both containing the *Campylobacter* selective

supplement containing three different lyophilized antibiotics (Merck, 1.02249).

The samples were incubated in a microaerobic atmosphere at 37 °C and 42 °C for a total of 48 h. The obtained pure *Campylobacter* cultures were further studied for production of cytochrome oxidase, catalase and hydrolysis of hippurate and indoxylacetate. The organisms with morphological, colonial and biochemical features of *Campylobacter* were differentiated via API Campy ® (bioMérieux, 20800).

RESULTS

Table 1 shows that *Campylobacter* positive were 114 or 76.2% out of all 185 samples, obtained throughout the slaughterhouse processing of poultry.

The carriership of *Campylobacter* in the different broiler chicken batches (herds) ranged from 60% on the skin surface of studied birds to 96% in the gut (Table 2).

Campylobacter organisms were isolated from the skin surface of birds at all technological stages of poultry carcass processing (Table 3). The percentage of *Campylobacter*-positive samples in the different batches varied between 64% and 80%. The lowest contamination was observed immediately after scalding (20%) whereas after evisceration, *Campylobacter* was detected in all specimens obtained from skin surfaces of the birds.

Table 1. *Campylobacter* positive samples in 185 specimens obtained during the slaughterhouse processing of poultry

	n	%
Campylobacter positive	141	76.2
C. jejuni	114	80.9
C. coli	27	19.1

Ninety-six percent of the specimens collected from the hands of personnel, participating in the evisceration were *Campylobacter*-positive (Table 4). The microorganism was also isolated from chilling tank water at the beginning (in 4 batches) and the end of the process (in 3 batches) (Table 4).

The biochemical differentiation of *Campylobacter* isolates showed that *C. jejuni* was the most commonly isolated species, followed by *C. coli*. One hundred and fourteen out of all 141 isolates were differentiated as *C. jejuni* (80.9%) and 27 – as *C. coli* (19.1%) (Table 1).

The further differentiation of the 114 *C. jejuni* isolates into subspecies revealed that the highest incidence was that of *C. jejuni subsp. jejuni* (69.3%) followed by *C. jejuni subsp. doylei* (30.7%). The former subspecies was encountered more frequently after defeathering (12.3%) whereas the contamination with the latter one was the highest after evisceration (7.9%).

DISCUSSION

Our data showed that 76.2% of studied samples from the poultry slaughterhouse were *Campylobacter* positive. Positive samples were detected at all technological stages, including the chilled poultry carcass. At some processing stages, the contamination with *Campylobacter* could be reduced, but the complete elimination of the agent could not be achieved. The percentage of positive samples obtained from skin surfaces of studied batches at the various technological stages varied in a relatively wide range and attained 100%. Our data are similar to those of Ono and Yamamoto (1999) that reported presence of *Campylobacter* at all stages of the

Table 2. Carriership of *Campylobacter* in broiler chickens at the age of slaughtering, %

Batch	Skin surface[1]			Caecum[2]		
	Number of samples	*Campylobacter* positive		Number of samples	*Campylobacter* positive	
		n	%		n	%
I	5	2	40	5	4	80
II	5	4	80	5	5	100
III	5	4	80	5	5	100
IV	5	4	80	5	5	100
V	5	1	20	5	5	100
Total	25	15	60	25	24	96

[1] samples obtained during bleeding; [2] samples obtained during evisceration.

Table 3. Incidence of *Campylobacter* throughout the slaugherhouse processing of poultry

Processing stage	Number of samples	*Campylobacter* positive	Batch				
			I %	II %	III %	IV %	V %
Bleeding	25	15 (60%)	40	80	80	80	20
Scalding	25	5 (20%)	20	20	20	20	20
Plucking	25	23 (92%)	100	60	100	100	100
Evisceration	25	25 (100%)	100	100	100	100	100
Chilling	25	18 (72%)	60	60	60	100	80
Total	125	86 (68.8%)	64	64	72	80	64

Table 4. Incidence of *Campylobacter* in specimens from personnel hands and water at the start and at the end of the chilling tank

	Number of samples	*Campylobacter* positive	
		n	%
Personnel	25	24	96%
Water – at the start	5	4	80%
Water – at the end	5	3	60%

poultry slaughtering process. In two batches, the percentage of *Campylobacter*-positive specimens obtained during the processing was exceptionally high: 80% and 72% for batches IV and III respectively. The broiler chickens from both batches showed a 100% carriership of the bacterial agent in the gut and 80% on the skin surface. That high initial contamination at the beginning of the processing is a prerequisite for the higher contamination at the different technological stages.

According to literature data, *Campylobacter* is observed in all birds during the bleeding (Mead *et al.*, 1995; Berrang and Dickens, 2000). Other studies report only 20% positive samples (Jones *et al.*, 1991). Compared to those reports, our data about

the incidence of isolation could be interpreted as intermediate.

According to the studies of Slavik *et al.* (1994), the scalding at 56 °C resulted in the highest reduction effect, but the counts of *Campylobacter* on skin surface of birds were still significant (lg 3.39). This finding is supported also by our results, when the water temperature was 58 °C. It is assumed that bacteria surviving the scalding, including Campylobacters, enter the plucking machine and the risk of cross contamination of other birds increases. Ono and Yamamoto (1999) affirmed a reduction of *Campylobacter* isolation after scalding and an elevation after defeathering. In our study, the percentage of positive samples after defeathering corresponds to the observation of other authors that defathering is an important critical point, creating prerequisites for cross contamination.

Another critical point is the evisceration. The specimens obtained from the body cavity and skin surface of birds after evisceration as well as from the working surfaces of eviscerator could be up to 100% positive (Berndtson *et al.*, 1996). Our experiment also confirmed this finding.

The personnel of poultry slaughterhouses is also a critical point with regard to the rsik of cross contamination of poultry carcasses (Wedderkopp *et al.*, 2000; Slader *et al.*, 2002). Our data allowed us to assume this evaluation too.

Stern *et al.* (2003) reported that 62% of birds were contaminated with *Campylobacter* after the water chilling, the level of contamination being between lg 4.97 and lg 2.95 microbial cells per bird during the various months of the year. Although relatively higher, the percentage obtained by us (72%) is similar. At the same time, it coresponds to that of water samples from the chilling tank. According to some studies, *Campylobacter* organisms could penetrate into feather follicles that provide them protection and conditions for surviving (Mead *et al.*, 1995). The same investigators show that even when water chlorine content was high (40 ppm), the final reduction effect upon *Campylobacter* is insignificant.

Our data about the diversity of *Campylobacter* spp. are identical to the data reported by Mea *et al.* (1995); Ono and Yamamoto (1999). The percentage of *C. jejuni subsp. jejuni* (69.3%) was relatively lower than that reported by Atabay and Corry (1997) in all birds in the poultry slaughterhouse. At the same time, the incidence of *C. jejuni subsp. doylei* isolates in our experiment (30.7%) was higher than the respective percentage in the studies of Ridsdale *et al.*, (1998) – only in 10% of studied samples.

CONCLUSION

During the slaughterhouse processing of poultry, the contamination with *Campylobacter* was lower at some technological stages whereas at other stages, a higher bacterial contamination was present. The primary critical points were the defeathering, the evisceration and the water chilling. The conditions at the poultry slaughterhouse allowed the survival and the presence of *Campylobacter* during the processing of poultry carcasses. Thus, the raising of *Campylobacter*-free broiler herds is essential for the restriction of microbial contamination in poultry slaugherhouses.

REFERENCES

Altekruse, S. F., N. J. Stern, P. I. Fields and D. L. Swerdlow, 1999. *Campylobacter je-*

juni – an emerging foodborne pathogen. *Emerging Infectious Diseases*, **5**, 28–35.

Atabay, H. I. and J. E. L. Corry, 1997. The prevalence of Campylobacters and arcobacters in broiler chickens. *Journal of Applied Microbiology*, **83**, 619–626.

Brendtson, E., M. L. Danielsson-Tham and A. Engvall, 1996. *Campylobacter* incidence on a chicken farm and the spreads of *Campylobacter* during the slaughter process. *International Journal of Food Microbiology,* **32**, 35–47.

Berrang, M. E. and J. A. Dickens, 1999. Presence and level of *Campylobacter* on broiler carcasses throughout the processing plant. *Journal of Applied Poultry Researches*, **49**, 321–337.

Bryan, F. L. and P. Doyle, 1995. Health risks and consequences of *Salmonella* and *Campylobacter jejuni* in raw poultry. *Journal of Food Protection*, **58**, 326–344.

Corry, J. E. L. and H. I. Atabay, 2001. Poultry as a source of *Campylobacter* and related organisms. *Journal of Applied Microbiology*, **90**, 96S–114S.

Engberg, J., S. Livingstone, W. On, C. S. Harrington and P. Smidt, 2000. Prevalence of *Campylobacter*, *Arcobacter*, *Helicobacter* and *Sutterella* spp. in human fecal samples as estimated by a revaluation of isolation methods for Campylobacters. *Journal of Clinical Microbiology*, **38**, 286–291.

Jacobs-Reitsma, W. F., N. M. Bolder and R. W. Mulder, 1994. Caecal carriage of *Campylobacter* and *Salmonella* in Dutch broiler flocks at slaughter. *Poultry Science*, **73**, 1260–1266.

Jones, F. T., R. C. Axtell, D. V. Rives, F. R. Scheideler, J. R. Tarver, R. L. Walaer and M. J. Wineland, 1991. A survey of *Campylobacter jejuni* contamination in modern broiler production and processing systems. *Journal of Food Protection*, **49**, 259–262.

Mead, G. C., W. R. Hudson and M. H. Hinton, 1995. Effect of changes in processing to improve hygiene control on contamination of poultry carcasses with *Campylobacter*. *Epidemiology and Infection*, **115**, 495–500.

Anonymous, 1997. Generic HACCP application in broiler slaughter and processing. *Journal of Food Protection*, **60**, 579–604.

Ono, K. and K. Yamamoto, 1999. Contamination of meat with *Campylobacter jejuni* in Saitama, Japan. *International Journal of Food Microbiology*, **47**, 211–219.

Padungtod, P., R. Hanson, D. L. Wilson, J. Bell, J. E. Linz and J. B. Kaneene, 2002. Identification of *Campylobacter jejuni* isolates from cloacal and carcass swabs of chickens in Thailand by a 5´nuclease fluorogenic polymerase chain reaction assay. *Journal of Food Protection*, **65**, 1712–1716.

Rautelin, H. and M. L. Hanninen, 2000. Campylobacters the most common bacterial enteropathogens in the Nordic countries. *Annals of Medicine*, **32**, 400–445.

Ridsdale, J. A., H. I. Atabay and J. E. L. Corry, 1998. Prevalence of Campylobacters and arcobacters in ducks at the abattoir. *Journal of Applied Microbiology*, **85**, 567–573.

Slader, J., G. Domingue, F. Jorgensen, K. McAlpine, R. J. Owen, F. J. Bolton and T. Humphrey, 2002. Impact of transport crate reuse and of catching and processing on *Campylobacter* and *Salmonella* contamination of broiler chickens. *Journal of Applied Environmental Microbiology*, **68**, 713–719.

Slavic, M. F., J. W. Kim, M. D. Pharr, D. P. Raben, S. Tsai and C. M. Lobsinger, 1994. Effect of trisodium phosphate on *Campylobacter* attached to post-chill chicken carcasses. *Journal of Applied Microbiology*, **57**, 324–326.

Stern, N. J., M. R. S. Clavero, J. S. Bailey, N. A. Cox and M. C. Robach, 1995. *Campylobacter* spp. in broilers on the farm and after transport. *Poultry Science*, **74**, 937–941.

Stern, N. J., K. L. Hiett, G. A. Alfredsson, K. G. Kristinsson, J. Reiersen, H. Hardardot-

tir, H. Briem, E. Gunnarsson, F. Georgsson, R. Lowman, E. Berndtson, A. M. Lammerding, G. M. Paoli and M. T. Musgrove, 2003. *Campylobacter* spp. in Icelandic poultry operations and human disease. *Epidemiology and Infection*, **130**, 23–32.

Wedderkopp, A., E. Rattengorg and E. Madden, 2000. National surveillance of *Campylobacter* in broilers at slaughter in Denmark in 1998. *Avian Diseases*, **44**, 993–999.

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