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Determination of *Campylobacter* spp. in poultry slaughterhouses and poultry meat

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ABSTRACT

The aim of this study was to determine the presence of *Campylobacter* spp. in poultry carcasses in poultry slaughterhouse areas and in poultry meat samples from the examined poultry slaughterhouses, following its placement on the market. The research was conducted in spring 2007 at 5 poultry slaughterhouses in Međimurje County, followed by an examination of poultry meat placed on the market for sale. A total of 75 poultry meat swabs, 15 samples of carcass cooling water and 15 samples of poultry meat collected in retail shops were examined. None of the samples collected in the premises of poultry slaughterhouses was found positive for *Campylobacter* spp. In 10 samples of poultry meat collected at the retail level the presence of *Campylobacter jejuni* and *Campylobacter coli* was determined.

Key words: *Campylobacter* spp., poultry slaughterhouses, poultry meat

Introduction

Campylobacter spp. are Gram-negative microaerophilic bacilli, having a somewhat curved, rod-like appearance, with two cells forming a short chain resembling seagull wings (HUNT, 1992). Campylobacter is characterised by flagella-mediated corkscrew motility. It is a typical microaerophilic microorganism with respiratory type metabolism, requiring 3-15% O_2 and 3-5% CO_2. These microorganisms do not produce acids from carbohydrates and use amino acids as a source of energy (HOLT et al., 2000). The cells can survive 2-4 weeks in a humid environment at 4 °C or 2-4 months at -20°C, while at room temperature the survival time is limited to a few days (BLASER et al., 1980). They are thermophilic organisms showing the best growth at 42 °C and include: *C. jejuni*, *C. coli* and *C. lari*. *Campylobacter* can be found in the reproductive organs, intestinal

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tract and oral cavity of humans and animals, and under favourable conditions may cause disease (HOLT et al., 2000). Sources of infection for poultry include feed, water and litter. The birds usually do not show any signs of disease, but bacteria from the intestines can contaminate carcass surfaces during evisceration in the slaughterhouse and subsequently may be transmitted to humans (NAGLIĆ et al., 2005).

Campylobacter spp. are highly infectious microorganisms and the most frequent causative agents of intestinal infections in humans in the USA and other industrially developed countries. The incidence of food borne *Campylobacter* infections has increased significantly in recent years and outnumbered salmonelloses (KELLER et al., 2007). Among the isolates causing gastroenteritis, the most frequently isolated species was *C. jejuni* (93%), followed by *C. coli* (7%), *C. lari* and *C. hyointestinalis* (BRAWN et al., 2004). Poultry meat and poultry products are the most important sources of the infection (RICHARDSON et al., 2005).

The infectious dose is very low, up to 500 CFU/g, and depends on age and general physical conditions, and in children this dose may be significantly lower (KOTHARY and BUBA, 2001). The most common clinical symptoms of campylobacteriosis include diarrhoea, in children frequently blood stained (30%), abdominal pain, fever, nausea and sometimes vomiting. The symptoms may range from mild gastroenteritis to severe disease and acute appendicitis. Complications delaying the recovery include reactive arthritis which may lead to Guillain-Barré Syndrome, acute neuromuscular paralysis. Systemic infections may lead to sepsis, endocarditis or meningitis. The incubation period is 1-10 days, on average 2-5 days (ANONYM., 1997).

The purpose of this study was to determine the risk in manipulation from slaughterhouse to the market.

Materials and methods

Swabs were taken from poultry carcasses at different processing points, between individual cooling stages. The temperature of the poultry carcasses decreased by 5 °C on average with each cooling stage. So the initial carcass temperature was 30 °C and fell to approx. 15 °C after cooling in ice water tanks. After the rapid cooling (chilling) process, the poultry carcasses were kept in a cold store at 2 °C.

In each test tube containing the smear, 10 mL of Buffered Peptone Water (1.07228. MERCK) was added for preliminary enrichment. The samples were then incubated at 37 °C for 24h.

Subsequently, 1 mL of pre-enriched and incubated samples was added to 9 mL of Bolton Enrichment Broth (1.00068.MERCK) to which Bolton Selective Supplement (1.00079. MERCK) was added. These prepared samples were incubated at 41 °C for 24h.

After the incubation, the samples were streaked with a loop onto Campy Food ID Agar (43 471, bioMérieux) and incubated at 41 °C under microaerophilic conditions for 24-48 h. Microaerophilic conditions were created in an anaerobic incubator (96 127, bioMérieux) by applying a medium supporting microaerobic conditions (96 125, bioMérieux).

Water was collected from poultry carcass cooling tanks at 5 slaughterhouses. Average water temperature was 13 °C.

From each water sample 1 mL was added to 9 mL of Buffered Peptone Water for preliminary enrichment and incubated at 37 °C for 24h.

After the incubation, 1 mL of pre-enriched sample was added into 9 mL of Bolton Selective Enrichment Broth and incubated at 41 °C for 24h, then streaked onto Campy Food ID Agar and incubated at 41 °C for 24-48h under microaerophilic conditions.

Poultry meat. Poultry meat samples and poultry carcasses were collected from cool places at retail shops. 10 g of each, were added to 90 g Buffered Peptone Water for preliminary enrichment and then incubated at 37 °C for 24h.

Subsequently, 1 mL of pre-enriched sample was added to 9 mL of Bolton Selective Enrichment Broth and incubated at 41 °C for 24h.

Following the incubation, the samples were streaked with a loop onto Campy Food ID Agar and incubated at 41 °C for 24-48h under microaerophilic conditions.

Suspect colony identification. Suspect colonies on Campy Food ID Agar were small, and dark red to orange-red colour with a metallic sheen, staining Gram negative and forming characteristic shapes resembling seagull wings. The staining is more intense in carbol fuchsin, and oxidase enzyme is produced. Identification with sodium hippurate (8.20648. MERCK) and ninhydrin solution is performed by adding the colonies with a loop into 2 mL of 1% sodium hippurate solution (8.20648. MERCK) and incubating them at 37 °C for 2h. Then 1 mL of 3,5% ninhydrin solution (1.06762. MERCK) is added and the sample is incubated at 37 °C for 1-2h. Blue staining indicates the presence of *C. jejuni* while absence of staining confirms the presence of *C. coli*.

The method used for isolation was a method certified in accordance with HRN ISO 10272-1:2006.

Results

A total of 75 samples were examined, consisting of the swabs taken in 5 controlled slaughterhouses from poultry carcass surfaces before cooling, between individual cooling stages and after the cooling. Apart from a large total count of aerobic mesophilic bacteria and the isolation of *E. coli* and *E. faecalis*, no *Campylobacter* spp. were determined in any of the examined samples.

Also, 15 water samples collected from the carcass cooling tanks at different cooling stages were examined. In particular, 3 samples from 3 different tanks were collected at each slaughterhouse. None of these samples was found positive for *Campylobacter* spp.

In addition, 15 poultry meat samples, from the same manufacturers and at the same period, were collected at the retail level. A total of 10 samples of poultry meat were found positive for *Campylobacter* spp. (66,6%) of which *C. jejuni* was isolated from 6 samples (40,0%), and *C. coli* from 4 samples (26,6%) of poultry meat.

Table 1. Number of *Campylobacter* spp. isolates in smears, water and poultry meat samples

	Number of samples	Positive findings (%)		Total (%)
		C. jejuni	*C. coli*	
Swabs	75	-	-	-
Water	15	-	-	-
Meat	15	6 (40.0)	4 (26.6)	10 (66.6)
Σ	105	6 (6.3)	4 (4.2)	10 (10.5)

Discussion

Swabs taken from poultry carcasses were analysed because poultry skin is considered to be one of the main sources of this microorganism (MUSGROVE et al., 1997). Contrary to our findings, data from relevant literature indicate the extremely high contamination of the skin of poultry carcasses in slaughterhouses. Using the method of poultry skin swabs taken in slaughterhouses, *Campylobacter* spp. was isolated in 79% of poultry meat samples in Belgium. The same percentage of isolates was achieved in USA, in the swabs taken from poultry skin (BERRANG et al., 2001; JEFFREY et al., 2001).

Examination of cooling and rinsing water in New Zealand confirmed the presence of *C. jejuni* in 28.2% samples (TSUEI et al., 2007). In a similar examination of poultry carcass rinsing water in Brazil (FRANCHIN et al., 2005) *Campylobacter* spp. was determined in 25% of water samples, which is also contrary to our results.

Other authors report a very high percentage of isolated *Campylobacter* spp. from poultry carcasses after cooling i.e. 55.1% and 78.5% isolated from poultry carcasses in slaughterhouses (SON et al., 2007). As many as 100% poultry carcasses were found invaded at the end of processing at a poultry slaughterhouse in Hungary (JOZWIAK et al., 2006). The source of contamination were the hands of the workers and the environment. As regards the poultry meat at retail sale level, *Campylobacter* spp. were isolated from 49.5% of samples in Spain (DOMINGUEZ et al., 2002), 31% in Vietnam (LUU et al., 2006), 94% in North Ireland (MOORE et al., 2002) and 45.9% in Germany (ATANASSOVA and

RING, 1999). The findings of the examination undertaken in Croatia (KOZAČINSKI et al., 2006) are quite opposite, when no positive poultry meat sample was found positive.

The purpose of this examination was to identify the connection between the contamination of poultry meat and carcass cooling water in slaughterhouses. Actually, the water was believed to be the source of poultry meat contamination with *Campylobacter spp*.

After the completed examination, it was established that the cooling water was not a source of poultry meat contamination. Manufacturing and storage practices and conditions at slaughterhouses also were not the source of contamination. The contamination occurred at another handling stage, in transport and the point of sale.

The environment-air, water, soil, could be the main source of *Campylobacter* spp. and its further transmission to poultry meat. Drinking water may be also one of possible sources of contamination. This assumption is supported by 9.1% positive findings in drinking water (MOORE et al., 2002) and 41.7% positive findings in other water samples. Other possible sources of contamination include the air (BULL et al., 2006), workers' tools, accessories and hands, due to inadequate handling or temperature (WHITE et al., 1997).

Considering that the relevant legislation provides that food intended for human consumption shall be under strict control at each stage of manufacture, processing, transport and sale in order to meet the food safety requirements „from stable to table", a disruption of the continuity of microbial safety is observable. Therefore, it is indispensable to continue these examinations in terms of control of critical points (HACCP) from the point of manufacture to the point of sale, in order to eliminate the weak points that lead to poultry meat contamination.

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SAŽETAK

Cilj istraživanja bio je utvrditi prisutnost *Campylobacter* spp. na trupovima peradi u klaonicama peradi i u uzorcima mesa iz klaonica peradi koje je stavljeno u prodaju. Istraživanje je provedeno u proljeće 2007. godine na 5 klaonica peradi na području Međimurske županije. Pregledano je 75 obrisaka mesa peradi, 15 uzoraka vode za hlađenje trupova te 15 uzoraka mesa peradi u prodaji. Ni u jednom uzorku iz klaonice nije ustanovljena prisutnost *Campylobacter* spp. Kod 10 uzoraka mesa peradi iz prodaje ustanovljene su vrste *Campylobacter jejuni* i *Campylobacter coli.*

Ključne riječi: *Campylobacter* spp., klaonice peradi, meso peradi