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Marek SELWET, Mariola GALBAS[1], Andrzej BORKOWSKI[2], Tomasz CŁAPA, Filip PORZUCEK[1]

THE EFFECT OF CHILLING AND FREEZING OF POULTRY MEAT IN THE PRESENCE OF *CAMPYLOBACTER JEJUNI*

WPŁYW SCHŁADZANIA I ZAMRAŻANIA MIĘSA DROBIOWEGO NA WYSTĘPOWANIE *CAMPYLOBACTER JEJUNI*

Department of General and Environmental Microbiology, Poznań University of Life Sciences, Poland
[1]Department of Biochemistry and Biotechnology, Poznań University of Life Sciences, Poland
[2]Faculty of Geology, University of Warsaw, Poland

Streszczenie. Niewłaściwie przechowywane mięso drobiowe w dużym stopniu może zostać skażone bakteriami z rodzaju *Campylobacter* spp. Schłodzenia i zamrażanie mięsa może być jedną z przyczyn zahamowania wzrostu tych bakterii na produktach mięsnych. Celem prowadzonych badań była kontrola wpływu niskich temperatur 4ºC i –18ºC na częstotliwość występowania tych bakterii w mięsie drobiowym. Próbki mięsa drobiowego (n = 450) były pobierane losowo z wybranych ubojni. Identyfikacji *C. jejuni* dokonano przy zastosowaniu testu Api Camy i reakcji PCR. Stwierdzono, że schładzanie spowodowało obniżenie występowania *C. jejuni* w surowym mięsie drobiowym o 90,3%. Mrożenie mięsa wyeliminowało w 100% skażenie bakteriami *C. jejuni*. Podsumowując można stwierdzić, że chłodzenie mięsa nie daje gwarancji całkowitej eliminacji bakterii *Campylobacter* spp.

Key words: *Campylobacter jejuni*, freezing, poultry meat.
Słowa kluczowe:*Campylobacter jejuni*, mięso drobiowe, zamrażanie.

INTRODUCTION

Meat products to be consumed by humans should be safe to use. Although the quality of these products has improved, there is still high incidence of bacterial poisonings and contaminations. This situation may be caused by the lack of hygiene, production practice and failure to abide by the HACCP standards (Pałkowska 2013). Both saprophytic and pathogenic microorganisms may be present in raw meat and their presence may result from primary or secondary contaminations (Danyluk and Pyrcz 2012). Inappropriate storage of poultry meat may cause its contamination with *Campylobacter* spp. bacteria. The commonness of *C. jejuni* e.g. in poultry meat, results from the presence of these bacteria in animals' alimentary tract. Chilling or freezing meat even to a temperature of –18ºC is a good method limiting the incidence of these pathogens (Hać-Szymańczuk 2012). It is necessary to remember that microorganisms develop at a faster rate in chilled food stored under the conditions of some temperature fluctuations than in frozen food (Piekarska 2012).

Corresponding author – Adres do korespondencji: Marek Selwet, Department of General and Environmental Microbiology, Poznań University of Life Sciences, Szydłowska 50, 60-656 Poznań, Poland, e-mail: marek.selwet@gmail.com

The aim of the study was to determine the influence of chilling and freezing meat acquired from slaughterhouses on the incidence of *C. jejuni* bacteria in the product. The analysis was conducted on whole poultry carcasses or their individual parts, which were products for sales.

MATERIAL AND METHODS

Poultry meat Poultry meat samples (n = 450) were collected at random from selected slaughterhouses in Masovian Voivodeship. The control sample was fresh meat which neither had been chilled in water nor frozen after slaughter. It was stored at 20ºC. The other meat samples were transported in containers maintaining temperatures of 4ºC and –18ºC. All the meat samples came from the poultry which had been examined before slaughter and approved for slaughter. All the samples were *Campylobacter* spp. carriers. The samples were analysed microbiologically within 24 h after collection. The samples were collected in winter between 1 December 2014 and 1 March 2015.

Campylobacter spp. isolation (Szczepańska et al. 2007). Transport swabs and meat samples were placed in 100 ml of liquid Preston medium (with ram's blood and Preston *Campylobacter* Selective Supplement added (Oxoid)). The bacterial culture was incubated under microaerophilic conditions (8% O_2, 7% CO_2). Next, 100 µl of the culture was placed on a bacteriological filter (pore diameter 0.65 µl). The filter was placed on the surface of Karmali agar (Oxoid) and CCDA agar (Oxoid).

After 24 hours of incubation at a temperature of 37ºC under microaerophilic conditions (Anaerocult C, Merck) the filters were removed and the medium was incubated for 48 hours under identical conditions.

Campylobacter bacteria were initially identified from Gram-negative colonies of catalase-positive bacteria. The API Campy test was applied (bioMérieux).

The PCR was conducted according to the procedure given by Szczepańska et al. (2007) and Wang et al. (2002). In order to identify the bacteria 25 µl of the reaction mixture was used. The mixture was composed of 2.5 µl 10 x PCR buffer, 200 µM dNTP Mix, 20 mM $MgCl_2$, primer concentration: 0.5 µM *C. jejuni*, 1 µM *C. coli*, 0.2 µM 23S rRNA, 1.25 U *Taq* DNA Polymerase, 2.5 µl DNA. The primer sequence for *C. jejuni* was as follows (product volume: 323 bp): CJF 5'-ACTTCTTTATTGCTTGCTGC-3', CJR 5'-GCCACAACAAGTAAAGAAGC-3'. *C. coli* (product volume 126 bp): CCF 5'-GTAAAACCAAAGCTTATCGTG-3', CCR 5'-TCCAGCAATGTGTGCAATG-3'. 23S rRNA (product volume 650 bp): 23SF 5'-TATACCGGTAAGGAGTGCTGGAG-3', 23SR 5'-ATCAATTAACCTTCGAGCACCG-3'. The reaction was carried out in a PerkinElmer thermal cycler. Initial denaturation at 95ºC for 6 minutes. It was followed by 30 cycles, where each cycle consisted of initial denaturation at 95ºC for 0.5 minutes, annealing primers at 59ºC for 0.5 minutes and extension at 72ºC for 0.5 minutes.

The products were analysed by means of electrophoresis in 1.5% agarose gel with ethidium bromide. *C. jejuni* ATCC 33560 (DSMZ Germany) was used as a reference strain.

RESULTS AND DISCUSSION

A total of 450 poultry meat samples were analysed. 79 (17.5%) of them proved to be contaminated with *C. jejuni* (Table 1). Nur Ilida and Faridach (2012) observed greater contamination of fresh, chilled and frozen poultry carcases with *C. jejuni* – 57 isolates acquired from 151 samples, i.e. 37.7% of the samples were contaminated. In our study the greatest number of positive samples was identified in fresh carcases, which had not been chilled or frozen. This could have resulted from the physiology of *C. jejuni*. Being thermophilic bacteria, their optimal growth temperature is 37–42ºC and their growth may be inhibited at lower temperatures than 30ºC (Saumya and Bryan 2004).

Table. 1. Number of samples analysed
Tabela 1. Liczba analizowanych próbek

Sample Próbka	Number of samples Liczba próbek	Number of positive samples Liczba próbek pozytywnych
Raw chicken meat (fresh) Surowe mięso drobiowe (świeże)	150	72
Raw chicken meat (chilled) Surowe mięso drobiowe (schłodzone)	150	7
Raw chicken meat (frozen) Surowe mięso drobiowe (zamrożone)	150	0
Total Suma	450	79

As far as fresh meat is concerned (n = 150), as much as 48% of the samples were contaminated with *C. jejuni* (Table 2). The contamination was observed both in whole poultry carcases and in individual portions. Meat cutting residues, thighs and whole carcases proved to be the most contaminated. Rodrigo et al. (2005) found contamination with *Campylobacter* in 84% of carcases after slaughter. This percentage was greater than in our study. Before slaughter the authors tested carcases for *Campylobacter* spp. As it turned out, 80% of cloacal swabs were contaminated with these bacteria. This observation proves that the slaughter process does not reduce the contamination of carcases.

Table 2. The presence of *C. jejuni* on fresh raw polutry meat
Tabela 2. Występowanie *C. jejuni* na świeżym mięsie drobiowym

Sample Próbka	*C. jejuni* positive samples / total no of samples Liczba próbek pozytywnych *C. jejuni* / ogólna liczba próbek	% of *C. jejuni* positive samples % próbek pozytywnych *C. jejuni*
Whole chicken Cały kurczak	26/49	53.1
Fillet Filet	4/17	23.6
Wings Skrzydełka	7/19	36.8
Thighs Uda	7/10	70.0
Liver Wątróbka	9/25	36.0
Gizzards Żołądki	4/10	40.0
Residues Pozostałości	15/20	75.0
Total Suma	72/150	

When poultry meat was chilled to a temperature of 4ºC, the number of samples contaminated with *C. jejuni* dropped to 4.6%, as compared with the carcases which were not chilled (Table 3). However, there are reports that chilling meat has no influence on reduced contamination of poultry carcases with *Campylobacter* spp. Rob et al. (2003) indicated that chilling itself did not guarantee safe storage of poultry meat. They observed that the survival rate of *C. jejuni* in a refrigerator at a temperature of 2ºC was greater than at room temperature (20ºC). However, Piekarska (2012) reported that temperatures ranging from 0ºC to 4ºC were the best for storage and transport of poultry meat and offal. Additionally, Danyluk and Pyrcz (2012) noted that raw material stored at 0ºC perished at a three times slower rate than at 5ºC. The results of our study point to the minimal contamination of poultry meat with these bacteria, but nevertheless the bacteria were present. This might confirm the research cited by Nur Ilida and Faridach (2012), who observed that *C. jejuni* maintained their physiological activity for a few weeks even at a temperature of 4ºC. Once again, meat cutting residues and thighs proved to be the most contaminated with the bacteria. The presence of *C. jejuni* was not observed on whole chicken carcases.

Table 3. The presence od *C. jejuni* on chilled raw polutry meat
Tabela 3. Występowanie *C. jejuni* na schłodzonym mięsie drobiowym

Sample Próbka	*C. jejuni* positive samales / total no of samples Liczba próbek pozytywnych *C. jejuni* / ogólna liczba próbek	% of *C. jejuni* positive samples % próbek pozytywnych *C. jejuni*
Whole chicken Cały kurczak	0/50	0.0
Fillet Filet	1/30	3.3
Wings Skrzydełka	1/25	4.0
Thighs Uda	2/10	20
Liver Wątróbka	0/10	0.0
Gizzards Żołądki	0/10	0.0
Residues Pozostałości	3/15	20.0
Total Suma	7/150	

The analysis of 150 meat samples frozen at a temperature of –18ºC did not show the presence of *C. jejuni*. According to the guidelines provided by Rywotycki (2011), temperatures ranging from –18ºC to –33ºC are ideal for storage of poultry meat and offal.

The continuously growing demand for poultry meat increases the incidence of contaminations with *Campylobacter* spp. Appropriate microbiological quality of meat is largely determined by its storage temperature and hygienic standards in the place where it is acquired. Continuous monitoring at every stage of meat processing should increase the safety of potential consumers, whose awareness of the quality of products for consumption is constantly growing.

CONCLUSIONS

1. The research findings show that *Campylobacter* spp. bacteria may cause widespread danger due to their presence in poultry meat.
2. During the cutting of poultry carcases the meat becomes contaminated with *C. jejuni* bacteria.
3. Chilling meat to a temperature of 4ºC limits or eliminates the presence of *C. jejuni* on the surface of poultry meat.
4. The process of freezing carcases to a temperature of –18ºC completely eliminates the presence of *C. jejuni* in poultry meat.

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Abstract. Improperly stored poultry meat may be contaminated with bacteria of the *Campylobacter* spp. genus. Chilling and freezing meat may be one of the factors inhibiting the growth of these bacteria on meat products. The aim of this study was to check the influence of low temperatures 4°C and –18°C on the frequency of occurrence of these bacteria in poultry meat. Samples of poultry meat (n = 450) were collected at random from selected slaughterhouses. *C. jejuni* were identified using an API test and PCR reaction. Chilling was found to reduce the occurrence of *C.jejuni* in raw poultry meat by 90.3%. Freezing meat completely eliminated contamination with *C. jejuni* bacteria. To sum up, chilling meat does not guarantee total elimination of *Campylobacter* spp.