



*Original Contribution*

**EFFECT OF 30-DAY STORAGE AT MINUS 18°C ON  
CAMPYLOBACTER CONTAMINATION OF FROZEN  
JAPANESE QUAILS (COTURNIX COTURNIX)**

**T. Stoyanchev\*, I. Vashin, V. Rusev, D. Dinkov**

Department of Food Hygiene and Technology, Veterinary Legislation and Management, Faculty of Veterinary Medicine, Trakia University, Bulgaria

**ABSTRACT**

Studies on prevalence of *Campylobacter spp.* in quails, partridges and pheasants, as well as the contamination of poultry products shows contradictory results. This paper shows data for the presence of *Campylobacter* contamination in three batches of broiler quails and the effect of 30-day storage at minus 18°C on broilers. Sampled quails are of Faraon breed. *Campylobacter* was isolated from 7.8% of quails before freezing. *C. jejuni* was the most frequently isolated *Campylobacter* species (71.4%), followed by *C. coli* (28.6%). After 30-day storage *Campylobacter* was found only from 4.4% of quail samples, of which *C. jejuni* was isolated from 75% and *C. coli* from 25% of quails.

**Key words:** Food safety, Japanese quail, freezing, meat, skin surface, *C. jejuni*, *C. coli*.

**INTRODUCTION**

*Campylobacter* is the most common cause of bacterial gastroenteritis in humans and poultry products are considered the main sources of *Campylobacter* infections [1, 2, 3]. After *Campylobacter* appearance in poultry farm it could be found through the fattening period and isolated during the slaughter processing [4, 5]. During the latter conditions for cross-contamination arise in different phases where *Campylobacter* are transferred from one quail to another or from the alimentary tract to carcass surface. Subsequently *Campylobacter* is isolated from poultry products [6, 7] in both chilled and frozen broilers and poultry products [8, 9, 10, 11]. Because of lower consumption of game meat and meat from delicacy birds, they are conserved mostly frozen. Presence of *Campylobacter* in food products has attained high significance.

This paper is aimed at determining the effect of freezing and storage on *Campylobacter* contamination of broiler quails.

**MATERIALS AND METHODS**

Samples were collected from quails from Faraon breed. Quails were randomly chosen from three batches after slaughter and processing, all intended for commercial use. Ten broilers of each batch were tested for *Campylobacter*, first in the day before freezing and second in 30-day of storage at minus 18°C. Samples were collected from each broiler from skin surface, chest abdominal cavity and a part of pectoral muscles.

Samples were prepared in compliance with ISO 10272 – 1:2006 [12] that describes a horizontal method for the detection of *Campylobacter spp.* Samples were cultivated in enrichment broth with antibiotic selective supplement (Merck, 1.02249) and on selective *Campylobacter* agar, containing antibiotic supplement (Merck, 1.02249).

Samples were incubated in microaerobic conditions at 37°C and 42°C for 48 hours. Pure cultures of *Campylobacter* were tested subsequently for cytochrome oxidase, catalase and hydrolysis of hippurate and indoxylacetate. Bacteria with cell, colonial and biochemical characteristics of *Campylobacter spp.* were differentiated by API Campy® system (Bio Mérieux, 20800).

\* **Correspondence to:** T. Stoyanchev, Department of Food Hygiene and technology, veterinary legislation and Management, Faculty of Veterinary Medicine, Trakia University, Stara Zagora 6000, Bulgaria; Tel.: 00359-42-699540; E-mail: [todor.stoyanchev@uni-sz.bg](mailto:todor.stoyanchev@uni-sz.bg)

## RESULTS AND DISCUSSION

*Campylobacter* was isolated from 7.8% (7 of 90) of the samples of broiler quails on the day before freezing. Most frequently isolated was *C. jejuni*, followed by *C. coli*. *C. jejuni* was detected in 71.4% (5 of 7) and *C. coli* in

28.6% (2 of 7) of *Campylobacter* positive samples. The biggest number of *Campylobacter* positive samples was found in chest-abdominal cavity (13.3%), and lowest positive detection of *Campylobacter* was found in pectoral muscles (**Table 1**).

**Table 1.** Prevalence of *Campylobacter* spp. in broiler quail carcasses on the day before freezing

Samples	n	Campylobacter positive n (%)	Batches		
			I	II	III
			n (%)	n (%)	n (%)
Skin surfaces	30	2 (6.7%)	1 (10%)	1 (10%)	0
Chest-abdominal cavity	30	4 (13.3%)	1 (10%)	2 (20%)	1 (10%)
Breast muscle	30	1 (3.3%)	0	1 (10%)	0

In the second batch *Campylobacter* was isolated in samples from the broiler skin surface as well as in chest-abdominal cavity and muscles. In the third batch only one *Campylobacter* positive sample was detected.

Jeffrey et al., reported (2002) 30% prevalence of *Campylobacter* in cloaks of pigeons raised for meat. After evisceration of the pigeons, *Campylobacter* positive were only 13% of all samples on skin surface.

Musgrove et al. (2003) isolated *Campylobacter* in 1.0 CFU/ml from 58.3% of samples from carcass rinse samples.

Giacoboni et al. (1999) also showed that the consumer should take measures accordingly when preparing food at home. They studied 120 frozen chicken broilers from

stores in Argentina and detected 35.8% (43 of 120) *Campylobacter* positive samples.

On the 30<sup>th</sup> day after freezing only 4.4 % (4 of 90) of the samples were *Campylobacter* positive. Compared to the day before freezing we found a decrease in the number of *Campylobacter* positive samples. Biochemical differentiation showed the almost same proportion between *C. jejuni* and *C. coli*. *C. jejuni* were the more common isolates (75%), followed by *C. coli* (25%). The biggest number of *Campylobacter* positive samples was found on broiler skin surface (**Table 2**). The study showed only one *Campylobacter* positive result of all samples from chest-abdominal cavity and breast muscles.

**Table 2.** Prevalence of *Campylobacter* spp. in broiler quail carcasses on the 30<sup>th</sup> day of freezing

Samples	n	Campylobacter positive n (%)	Batches		
			I	II	III
			n (%)	n (%)	n (%)
Skin surfaces	30	2 (6.7%)	1 (10%)	1 (10%)	0
Chest-abdominal cavity	30	1 (3.3%)	0	1 (10%)	0
Breast muscle	30	1 (3.3%)	0	1 (10%)	0

In the second batch *Campylobacter* was isolated for only one broiler in samples from skin surface, chest abdominal cavity and muscles. In the third batch all samples were *Campylobacter* negative.

Results from the survey showed a decrease in the number of *Campylobacter* positive samples in the process of storage below zero degree temperature. The reduction process is less strong in the second batch, as most probably the level of contamination in there was higher compared to the other batches.

Various studies [14, 15, 9] presented evidence for the reduction effect of freezing on poultry bacterial contamination.

Dufrenne et al. (2001) found a level of *Campylobacter* contamination on chilled poultry of 10 to 50x10<sup>3</sup> CFU. In 34% of all chilled broilers there was contamination of over 1x10<sup>3</sup> *Campylobacter* cells whilst on the frozen meat only 9% of all showed contamination at the same level.

NACMCF paper (1994) reported that the values of *Campylobacter* isolation in frozen poultry meat are five times lower compared to the isolation values in chilled poultry meat.

Lee et al. (1998) tested the capability of *C. jejuni* to survive on skin surface at different temperatures and atmosphere in packing. Reduction effect of temperature conservation

at minus 70°C was significantly less strong compared to the effect of conservation at minus 20°C. Reduction of the number of *C. jejuni* at minus 70°C was  $\log_{10}2$  and after 5 days it remains almost constant till the end of the study (56 days). At minus 20°C it could be seen strong reduction ( $\log_{10}0.8$ ) till the end of the experiment. *C. jejuni* survives freezing better in atmosphere of carbon dioxide, and stronger reduction was reported in nitrogen atmosphere and vacuum.

### CONCLUSION

Presence of *Campylobacter* in frozen quail carcasses even at minus 18°C indicate that *Campylobacter* survives freezing and defrosting successfully and remains to be a risk factor for the consumers. That underscores the need for hygiene measures to decrease the risk in following stages of processing as kitchen and heat processing of quails.

### REFERENCES

1. Stern, N.J., Clavero M.R.S., Bailey J.S., Cox N.A. and Robach M.C., *Campylobacter* spp. in broilers on the farm and after transport. *Poultry Science*, 74, 937-941, 1995.
2. Altekruze, S.F., Stern N.J., Fields P.I. and Swerdlow D.L., *Campylobacter jejuni* – an emerging foodborne pathogen. *Emergency Infection Diseases*, 5, 28-35, 1999.
3. Corry, J.E.L. and Atabay H.I., Poultry as a source of *Campylobacter* and related organisms. *Journal of Applied Microbiology*, 90, 96S-114S, 2001.
4. Bryan, F.L. and Doyle P., Health risks and consequences of *Salmonella* and *Campylobacter jejuni* in raw poultry. *Journal of Food Protection*, 58, 326-344, 1995.
5. Berrang, M.E., Buhr, R.J., and Cason, J.A., *Campylobacter* recovery from external and internal organs of commercial broiler carcass prior to scalding. *Poultry Science*. 79, 286 – 290, 2000.
6. Ono, K. and Yamamoto K., Contamination of meat with *Campylobacter jejuni* in Saitama, Japan. *International Journal of Food Microbiology*, 47, 211-219, 1999.
7. Denis, M., Refrégier-Petton J., Laisney M.-J., Ermel G., and Salvat G., *Campylobacter* contamination in French chicken production from farm to consumers. Use of a PCR assay for detection and identification of *Campylobacter jejuni* and *Campylobacter coli*. *Journal of Applied Microbiology*, 91:255 . 267, 2001.
8. Giacoboni, G., Puchuri, M. C. and Cerda, R., Thermotolerant *Campylobacter* in carcasses and retail poultry parts from different market places from La Plata city (Argentina). *Analecta Vet.*, 19, 51 – 54, 1999.
9. Dufrenne, J., Ritmeerster, W., Delfgou, E., Leusden, F. and Rob de Jones., Quantification of the contamination of chicken and chicken products in the netherlands with *Salmonella* and *Campylobacter*. *J. Food Protect.*, 64, 538 – 541, 2001.
10. Padungtod, P., Hanson R., Wilson D. L., Bell J., Linz J. E. and Kaneene J. B., 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, 2002.
11. Musgrove, M.T., Cox, N.A., Berrang, M.E. and Harrison, M.A., Comparison of weep and carcass rinses for recovery of *Campylobacter* from retail broiler carcasses. *Journal of Food Protection*, 66, 1720-1723, 2003.
12. ISO 10272 – 1:2006, Microbiology of food and animal feeding stuffs -- Horizontal method for detection and enumeration of *Campylobacter* spp. -- Part 1: Detection method, 2006.
13. Jeffrey, J.S., Tonooka, K.H. and Lozano, J., Prevalence of *Campylobacter* spp. from skin, crop and intestine of commercial broiler chicken carcasses at processing. *Poultry Science*, 80, 1390-1392, 2001.
14. National Advisory Committee on Microbiological Criteria for Foods. *Campylobacter jejuni/coli*. *J. Food Prot.* 57:1101 – 1121, 1994.
15. Lee, A., S. C. Smith, and P. J. Coloe.. Survival and growth of *Campylobacter jejuni* after artificial inoculation onto chicken skin as a function of temperature and packaging condition. *J. Food Prot.* 66:1609 – 1614, 1998.