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# The effect of slaughter operations on the contamination of chicken carcasses with thermotolerant *Campylobacter*

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**Abstract**

To evaluate the effect of specific slaughter operations on the contamination of broiler carcasses with naturally occurring thermotolerant *Campylobacter*, experiments were carried out in two Danish commercial slaughter plants (Plant I and Plant II). Six broiler flocks determined *Campylobacter* positive prior to slaughter were investigated at four sampling locations within each slaughter plant. Quantification of thermotolerant *Campylobacter* in 30 neck skin samples per flock per sampling location showed that the evisceration operation in Plant I led to a significant increase in the *Campylobacter* concentration of 0.5 $\log_{10}$ cfu/g in average, whereas no significant changes were observed during this operation in Plant II. Air chilling (Plant I) and water chilling (Plant II), both including a carcass wash prior to the chilling operation, caused similar, but significant reductions of 0.83 and 0.97 $\log_{10}$ cfu/g, respectively. In packed frozen chickens (Plant II) an additional reduction of 1.38 $\log_{10}$ cfu/g in average was obtained due to the freezing operation. In packed chilled chickens (Plant I), however, the number of thermotolerant *Campylobacter* per gram remained at the same level as after air chilling. Enumeration of thermotolerant *Campylobacter* in 30 intestinal samples per flock showed that in two of the six flocks examined the within flock colonization was very low (<3% and 27% positive samples). The remaining four flocks were colonized at percentages of 100 (three flocks) and 97 (one flock) and had intestinal mean counts ranging from 6.65 to 8.20 $\log_{10}$ cfu/g. A correlation between *Campylobacter* concentrations in intestinal content and on chicken carcasses after the defeathering operation was documented. This finding indicates that a reduction in the *Campylobacter* concentration on chicken carcasses may also be obtained by interventions aimed at reducing the concentration of *Campylobacter* in the intestines of the living birds.


*Keywords:* *Campylobacter*; Chicken; Carcass; Slaughter; Contamination; Freezing

## 1. Introduction

*Campylobacter* is the leading cause of zoonotic enteric human infections in most developed countries, including Denmark (WHO, 2001; WHO/FAO, 2001; Anonymous, 2004; European Commission, 2004). The main source of human *Campylobacter* infections is believed to be poultry meat based on the frequent occurrence of *Campylobacter* in this food type (Anonymous, 2004; European Commission, 2004) and the fact that case–control studies conducted world-wide repeatedly have identified handling of raw poultry and eating poultry products as important risk factors for sporadic campylobacteriosis (Schorr et al., 1994; Adak et al., 1995; Neal and Slack, 1997; Studahl and Andersson, 2000; Effler et al., 2001; Kapperud et al., 2003; Neimann, 2003; Friedman et al., 2004). The presence of the organisms on poultry meat derives from symptomless intestinal carriage in the live bird, which is rather frequent (Anonymous, 2004; European Commission, 2004). In 2003 for example, approximately 35% of the Danish broiler flocks harboured *Campylobacter* (Anonymous, 2004). During slaughter the intestinal content will inevitably contaminate the broiler carcasses (Oosterom et al., 1983b; Wempe et al., 1983; Izat et al., 1988; Cason et al., 1997; Berrang and Dickens, 2000; Stern and Robach, 2003), and since no process operations eliminate the organism, poultry meat is often

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contaminated with thermotolerant *Campylobacter* (Anonymous, 2004; European Commission, 2004). In 2003, 37% of the chicken meat and 42% of the turkey meat for sale at retail in Denmark were contaminated with *Campylobacter* (Anonymous, 2004). Consumer exposure to *Campylobacter* is unavoidable if chicken meat is not handled hygienically, i.e. by cross-contamination from raw poultry meat to ready-to-eat food and/or if the meat is not properly cooked before consumption (De Wit et al., 1979; Scott et al., 1982; De Boer and Hahne, 1990; Humprey et al., 2001; Kusumaningrum et al., 2003).

In Denmark, the strategy to control *Campylobacter* is based on the principles of food safety risk analysis (FAO/WHO, 1995; FAO/WHO, 1997), therefore risk management decisions have been anchored in the outcome of a quantitative risk assessment on *Campylobacter jejuni* in chicken meat (Christensen et al., 2001; Rosenquist et al., 2003). One result of this risk assessment was that a reduction of the concentration of *C. jejuni* on the meat was predicted to cause a significant decrease in the human *Campylobacter* cases. Therefore, one of the key elements in the control strategy has been to reduce the concentration of *Campylobacter* on the chicken carcasses during slaughter. To identify possible intervention sites in Danish slaughter plants, it is crucial to create knowledge about which slaughter operations that may contribute to increased faecal contamination and which operations that may reduce the contamination.

The aim of this study was to generate Danish data on the impact of different slaughter operations on the number of naturally occurring thermotolerant *Campylobacter* on broiler carcasses in order to identify targets for reducing interventions.

## 2. Materials and methods

### 2.1. Sampling

A total of 900 samples were collected from six broiler flocks slaughtered in two commercial Danish slaughter plants (Plant I and Plant II). The plants were selected due to their differences in scalding operation (Plant I, approximately 51 °C for 220 s; Plant II, approximately 58 °C for 160 s), chilling procedure (Plant I, air chilling at approximately 0 °C for 105 min; Plant II, counter flow water chilling, also called spin chilling at 16 to 4 °C for approximately 22 min), and end products (Plant I, chilled chickens, 4 °C; Plant II, deep-frozen chickens, −18 to −20 °C).

The flocks examined were one week prior to slaughter determined *Campylobacter* positive by analysis of three pools of five faecal droppings sampled at three different locations in the chicken houses using a standard procedure including sample pre-enrichment in Preston broth followed by isolation on modified Cefaperazone Charcoal Desoxycholate Agar (mCCDA) (Nordic Committee on Food Analysis, 1990).

To estimate the effect of specific slaughter operations on the *Campylobacter* contamination of the broiler carcasses, 30 samles of neck skin from each of the six flocks examined were collected at random immediately after the operations: defeathering, evisceration, air chilling (Plant I) or water chilling (Plant II), and packaging of either chilled (Plant I) or frozen carcasses (Plant II). At least 10 g neck skin was removed from each carcass using a sterile scalpel and placed in individual stomacher bags with filter. Additionally, for each flock, 30 samples of intestinal content were collected at random at the evisceration operation to estimate the within flock prevalence and the level of intestinal carriage of *Campylobacter*. At least 1 g intestinal material was removed from the intestine of each carcass using sterile utensils and placed in individual stomacher bags with filter. The staff at the plants collected all samples and sent them under cooling conditions (1–5 °C) to the analysing laboratories. In order to balance the experimental design and to overcome capacity limitations, half of the samples per sampling location per flock were sent to one laboratory and the remaining half to another laboratory. In total, four laboratories participated in the study.

### 2.2. Sample preparation

Neck skin samples of 10 g were stomachated for 120 s in 10 ml Buffered Peptone Water (BPW, Oxoid CM509) (dilution $10^0$), and intestinal samples of 1 g were stomachated for 120 s in 10 ml BPW (dilution $10^{-1}$). Then serial 10-fold dilutions were produced in BPW.

### 2.3. Quantification of thermotolerant Campylobacter

#### 2.3.1. Enumeration

Samples of 0.1 ml of appropriate dilutions of neck skin and intestinal material were surface spread-plated in duplicate onto dried, modified Abeyta-Hunt-Bark (mAHB) agar plates containing per litre: 40 g Bacto Heart Infusion Agar (Oxoid CM375), and 2 g Yeast Extract (Oxoid CM19) (U.S. Food and Drug Administration, 2001). After autoclaving at 121 °C for 15 min and cooling to 45 °C, each litre of AHB medium was supplemented with 4 ml sterile filtered Na-cefoperazone (Sigma C4292) (8.0 mg/ml), 4 ml Rifampicin (Sigma R3501) (2.5 mg/ml), two selective supplements (Oxoid SR84), and 10 ml triphenyl-tetrazoliumclorid (1%) (Bie and Berntsen LAB03920). Inoculated plates were incubated micro-aerobically for 48 h at 42 °C.

#### 2.3.2. Confirmation and estimation of colony forming units

After incubation the number of colony forming units (cfu) were counted. The presence of *Campylobacter* was confirmed by phase contrast microscopy (×1000) of minimum five colonies from each sample. On the basis of the confirmation, the number of thermotolerant *Campylobacter* per gram sample (=the concentration) was estimated.

### 2.4. Statistical analyses

The statistical analyses were carried out to identify 1) slaughter operations that significantly changed the *Campylobacter* concentration on the chicken carcasses, 2) differences in these changes between the two slaughterhouses, and 3) any relationship between the *Campylobacter* concentration in intestinal material and on the carcasses after defeathering.

Bacterial counts (cfu per g neck skin or intestinal material) were converted to $\log_{10}$ values to approximate the data to normal