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Campylobacter spp. and *Escherichia coli* contamination of broiler carcasses across the slaughter line in Danish slaughterhouses

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ABSTRACT

This study presents levels of *Campylobacter* spp. and *Escherichia coli* on broiler carcasses across the slaughter line in three fully automated Danish slaughterhouses with the aim to investigate differences in slaughter hygiene between the lines, correlation between concentrations of *E.coli* and *Campylobacter,* and finally, the relationship between *Campylobacter* counts in caeca and on chilled meat. In total, 15 commercial, indoor flocks were examined and from each flock 24 caecal samples and 24 carcass samples were collected from each of the control points after plucking (AP), after evisceration (AE) and after chilling (AC). Results showed distinct differences between slaughterhouses. For slaughterhouse I the contamination level was high AP and decreased AE while for slaughterhouse II the contamination level was low AP and increased AE. For slaughterhouse III the contamination level varied insignificantly across the processes. Results also showed differences in contamination levels of *E. coli* and *Campylobacter* between slaughterhouses. Mean counts of the two organisms increased or decreased concurrently from after plucking to after evisceration within slaughterhouses; however, after chilling counts of *E. coli* were reduced to a larger extent than counts of *Campylobacter*. This suggests that for processing of *Campylobacter* positive broilers *E. coli* may be used as an indicator of faecal contamination during the processing steps up to the point of chilling but not as an indicator of *Campylobacter* contamination of chilled broiler meat. A correlation was found, though, between the mean number of *Campylobacter* in caeca and the mean number of *Campylobacter* on broiler meat after chilling which means that the level in the gut at slaughter significantly impacts the level on the chilled meat. In conclusion, our data confirm that less faecal contamination throughout processing, and/or less *Campylobacter* in the gut at the point of slaughter will lead to less *Campylobacter* contamination on the meat and thereby improve food safety. Exchange of information between slaughterhouses on best hygiene practices and compliance with these is an option to reduce numbers of *Campylobacter* in broiler meat.

1. Introduction

Campylobacter is the most common cause of bacterial gastroenteritis in Denmark (Anonymous 2014), and worldwide (World Health Organization 2013). The number of human *Campylobacter* cases started to increase in the 1990 s, and in many countries the incidence continues to increase (European Food Safety Authority 2014). A few countries have been able to stop this increase (e.g. Denmark and Norway) (Jore et al., 2010; Rosenquist et al., 2009) or even turn the development into a decrease (e.g. Iceland and New Zealand) (Sears et al., 2011; Tustin et al., 2011).

In Denmark, *Campylobacter* control strategies have been in place since 2003 (Rosenquist et al., 2009). The primary focus of the control strategies has been and still is to control the pathogen in the broiler production chain, as it is generally accepted that broiler meat is the most important single source of foodborne *Campylobacter* infections in Denmark and generally in industrialized countries (World Health Organization 2013; European Food Safety Authority 2010b; Boysen et al., 2014).

Campylobacter contamination of broiler meat during slaughter and processing is a result of the spread of faecal material from the intestines of the birds to the carcasses. When a flock is fully colonized, the intestinal content may contain up to 10^8–10^9 *Campylobacter* cfu/g (Reich et al., 2008; Duffy et al., 2014; Koolman et al., 2014; Seliwiorstow et al., 2015). Studies have shown that plucking and evisceration are the two processes that contribute the most to *Campylobacter* contamination of broiler meat during processing (Seliwiorstow et al., 2015; Rosenquist et al., 2006; Nauta et al., 2009a), due to the potential of faecal contamination of the

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carcasses. The observation that faecal material is the source of contamination suggests that faecal bacteria like *E. coli* may be used as an indicator for *Campylobacter* contamination of broiler carcasses and broiler meat. However, only few studies have considered the correlation between *Campylobacter* and *E. coli* on carcasses during processing and broiler meat after processing. An Australian study found that the concentration of *Campylobacter* and *E. coli* was significantly correlated throughout processing; suggesting that *E. coli* may be useful as an indicator for reductions in *Campylobacter* concentration (Duffy et al., 2014).

Both observational studies (Reich et al., 2008; Seliwiorstow et al., 2015; Rosenquist et al., 2006) and *Campylobacter* risk assessments (Nauta et al., 2009; EFSA Panel on Biological Hazards (BIOHAZ) 2011; Nauta et al., 2007) suggest that high intestinal concentrations are correlated to higher levels of contamination of the meat and consequently to higher health risks for consumers. If a correlation between concentrations in caecal contents and on the meat after chilling is found, in Danish slaughter houses, this may facilitate risk assessment (Nauta et al., 2016).

This study investigates levels of *Campylobacter* spp. and *E. coli* on broiler carcasses across the slaughter line in three large Danish slaughterhouses with the aim to evaluate differences in slaughter hygiene performance between the production lines, any correlation between concentrations of *E. coli* and *Campylobacter*, and finally, the relationship between *Campylobacter* counts in caeca and on chilled meat.

2. Materials and methods

2.1. Sampling

Samples were collected at three fully automated broiler slaughterhouses, here referred to Slaughterhouse I, II and III, at three control points during processing; after plucking (AP), after evisceration (AE), and after chilling (AC). The chilling procedure differed between slaughterhouses; slaughterhouse I used forced air chilling, while slaughterhouses II and III used spray chilling. Samples were collected from commercial, indoor, broiler flocks detected positive for *Campylobacter* in sock samples collected seven to ten days before slaughter. All sampled flocks were slaughtered on different days. In total, 15 flocks were sampled; five flocks per slaughterhouse. From each flock, 24 samples were collected at each control point, selected at random throughout the whole flock; all samples were collected with appropriate intervals. Each sample consisted of one carcass from which one thigh with skin was cut off (left or right was not specified) for further analysis. The skin was cut from the thigh for analysis (app. 20 g). Additionally, 24 individual caeca were collected per flock; immediately after evisceration. The caeca were not matched with the sampled thighs. The samples were collected and analysed by the Danish Regional Veterinary and Food Authorities.

2.2. Microbial analysis

Sock samples were analyzed for the presence of *Campylobacter* by PCR. Preparation and detection were performed as described by Lund et al. (2003). Caecal samples were analyzed for *Campylobacter* while each thigh sample was analyzed for both *Campylobacter* and *E. coli*. Quantitative *Campylobacter* analysis was performed by direct plating according to the standard NMKL method 119; preparation, plating, incubation and interpretation is described in detail in the NMKL 119, 3. ed. protocol (Anonymous 2007). The lower limit of quantification for *Campylobacter* on skin samples was 10^1 cfu/g and 10^2 cfu/g for caecal samples.

E. coli on skin from thighs were enumerated on EC petrifilm plates (*E. coli*/Coliform Count Plates; 3M, Australia). Typical colonies were confirmed following standard procedures. The lower and upper limit of quantification for *E. coli* on skin samples were 10^1 and 10^5 cfu/g, respectively.

2.3. Data analysis

Bacterial counts (cfu per g skin or intestinal material) were $\log_{10}$ transformed. Some analytical results fell either below the detection limit (for *Campylobacter*; from one to seven samples in five of 15 flocks) or above (for *E. coli*; from one to 17 samples in eight of 15 flocks). To allow for the incorporation of these censored data, maximum likelihood estimation (MLE) was used to obtain estimates for the mean and the standard deviation for the concentrations ($\log_{10}$ cfu/g) for each broiler flock at each control point (Lorimer and Kiermeier, 2007) assuming a normal distribution of log transformed concentrations. MLE was performed assuming 100% within flock prevalence since sampling was carried out from flocks that were already positive for *Campylobacter* 7–10 days prior to slaughter and caecal counts of *Campylobacter* were all high.

Linear regression using PROC GLM was used to analyze the general linear model relationship between variables; with 'slaughterhouse', 'flock' and 'control point' as classification variables and numbers of *E. coli* and *Campylobacter* as continuous variables. Additionally, for the simple linear regression pairwise differences were derived with the LSMEANS statement using the PDIFF option. Analysis regarding correlation of *E. coli* and *Campylobacter* was based on the observed number in each sample, while analyses using mean numbers are based on MLE estimates.

Linear correlation between *Campylobacter* in caeca and on the chilled meat was further assessed using the PROC CORR statement including the Pearson product-moment correlation coefficient (PCC) to determine the degree of association between the variables. All analyses were performed using SAS 9.4 (SAS Institute Inc., Cary, NC, USA).

3. Results

The mean *Campylobacter* concentration ($\log_{10}$ cfu/g) in caecal samples varied between flocks, but the difference between slaughterhouses was non-significant ($P = 0.629$). The mean counts for the five flocks slaughtered in slaughterhouse I, II and III were 7.4 $\log_{10}$ cfu/g, 7.2 $\log_{10}$ cfu/g and 7.3 $\log_{10}$ cfu/g, respectively. Hence, the *Campylobacter* input to the slaughterhouses was not considered to be different and therefore it was assumed not to have influenced bacterial results across the slaughter line.

Results for *E. coli* and *Campylobacter* on thigh skins sampled across the slaughter line are presented in Table 1. Mean counts of the two bacteria increased or decreased simultaneously throughout the slaughter processes without considerable differences between flocks within slaughterhouses, except for the control point AC where counts of *E. coli* are reduced to a larger extent than counts of *Campylobacter*. Reductions of *E. coli* and *Campylobacter* AE-AC were 1.2, 1.0, and 0.4 $\log_{10}$ cfu/g and 0.1, 0.6, and 0.3 $\log_{10}$ cfu/g per slaughterhouse, respectively. Independent of the *E. coli* levels AE, the mean numbers of *E. coli* reached statistically comparable levels AC (Table 1), which was not observed for *Campylobacter* (Table 1).

Overall, distinct patterns were observed between slaughterhouses. For slaughterhouse I, contamination (*E. coli* and *Campylobacter*) decreased throughout processes, but the level of contamination was markedly higher at the first control point (AP) compared to slaughterhouse II and III. For slaughterhouse II, contamination increased at the second control point (AE), while the contamination level at slaughterhouse III only varied insignificantly across the processes.