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A comparison of fluctuations of *Campylobacter* and *Escherichia coli* concentrations on broiler chicken carcasses during processing in two slaughterhouses

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ABSTRACT

The causes of differences in *Campylobacter* and *Escherichia coli* concentrations on broiler chicken carcasses after chilling between slaughterhouses are not fully identified. Therefore, it is a challenge for slaughterhouses to comply with Process Hygiene Criteria for broiler meat.

The aim of the study was to identify which processing steps contribute to increases or decreases in *Campylobacter* and *E. coli* concentrations within and between two slaughterhouses. Identifying the processing steps with variable performance could explain the differences in bacterial concentrations after chilling between slaughterhouses.

Thermotolerant *Campylobacter* and *E. coli* concentrations on carcasses during broiler processing were measured during the summer period in 21 trials after bleeding, scalding, defeathering, evisceration and chilling.

In two slaughterhouses with comparable *Campylobacter* and *E. coli* concentrations in the incoming batches (after bleeding), the mean $\log_{10}$ concentrations are found to be significantly different after chilling. *Campylobacter* concentrations decreased by 1.40 $\log_{10}$ in Slaughterhouse 1 and by 1.86 $\log_{10}$ in Slaughterhouse 2, whereas *E. coli* decreased by 2.19 $\log_{10}$ in Slaughterhouse 1 and by 2.84 $\log_{10}$ in Slaughterhouse 2. Higher concentrations of *Campylobacter* and *E. coli* on carcasses after chilling were observed in Slaughterhouse 1 in which an increase in concentrations was observed after evisceration. The effect of processing on *Campylobacter* and *E. coli* concentrations in Slaughterhouse 1 did not differ between batches. In Slaughterhouse 2, the effect of processing on the concentrations of both bacteria varied over batches. Changes in *E. coli* concentration levels during processing were similar to *Campylobacter* except for defeathering. *E. coli* concentration significantly decreased after defeathering in both slaughterhouses, whereas *Campylobacter* increased in Slaughterhouse 2 and in Slaughterhouse 1 no significant changes were observed.

The patterns of increases and decreases in bacterial concentrations during processing are specific for each slaughterhouse. Inhomogeneous patterns potentially explain the differences in concentrations after chilling between slaughterhouses. Critical processing steps should be validated in each slaughterhouse by longitudinal studies and potentially based on *E. coli*. *E. coli* has a potential to be used as an indicator of processing hygiene, because the impact of most of the studied processing steps was similar as for *Campylobacter*.

1. Introduction

Campylobacter has remained the major gastrointestinal bacterial pathogen in humans in the European Union (EU) since 2005 (European Food Safety Authority, 2014). The annual number of campylobacteriosis cases was estimated at 9 million in the European Union (Havelaar et al., 2009). Broiler meat is recognised as a major source of human infections. An estimated 20–30% of cases of campylobacteriosis in EU may be attributed to the handling, preparation and consumption of broiler meat (European Food Safety Authority, 2010a). According to risk assessment studies, the most effective reduction of human infections in the short term could be achieved by reducing *Campylobacter* numbers in

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contaminated slaughtered batches (Nauta et al., 2009). Compliance of batches sold as fresh meat with a threshold value of 1000 or 500 CFU/g of neck and breast skin would reduce the health risk by more than 50% or even 90% (European Food Safety Authority, 2011). These risk assessment results drive the initiative to establish Process Hygiene Criteria (PHC) for broiler meat to stimulate further control measures aiming at reducing carcass contamination (European Food Safety Authority, 2012a). Moreover, the PHC could be used as a tool to classify slaughterhouses according to their capability to prevent or reduce hazards and as a tool to monitor risk and verify hygiene management in slaughterhouses (European Food Safety Authority, 2012a). Compliance to the PHC is, however, a challenge for the industry. In 20% of the tested batches in the European Union, the *Campylobacter* concentrations in neck and breast skin after chilling exceeded 1000 CFU/g (European Food Safety Authority, 2010b). In The Netherlands, 30% of all produced batches would not meet the threshold of none of 5 samples per batch exceeding 1000 *Campylobacter* CFU/g of breast skin after chilling (Anonymous, 2011; Swart et al., 2013). Differences in *Campylobacter* concentrations after chilling between slaughterhouses were identified in the baseline surveys (Anonymous, 2011; European Food Safety Authority, 2010b). However, the causes of the differences were not fully identified. *Campylobacter* concentrations change along the processing line with typically a decrease after scalding and chilling, an increase after defeathering and an increase or no change after evisceration (Berrang and Dickens, 2000; Izat et al., 1988; Klein et al., 2007; Oosterom et al., 1983; Rosenquist et al., 2006; Seliwiorstow et al., 2012; Tchórzewska et al., 2013). It has not been investigated whether these changes in concentrations are maintained at similar levels between batches and between slaughterhouses. Identifying the processing steps with variable performance within and between slaughterhouses could explain the differences in *Campylobacter* concentrations after chilling and thus improve the ability of the slaughterhouses to comply with potential Process Hygiene Criteria.

Setting a PHC based on *Escherichia coli* instead of on pathogenic bacteria has been proposed (European Food Safety Authority, 2012a; European Food Safety Authority, 2012b), because indicator microorganisms are recognised to reflect better the process hygiene than pathogenic microorganisms. The advantage of using *E. coli* to monitor processing performance is also related to easier, lower-cost, omnipresent enumeration techniques and frequent occurrence of *E. coli* on the carcasses that is not impacted by seasonality as in the case of *Campylobacter* (European Food Safety Authority, 2012a; Habib et al., 2012). Furthermore, targets based on *E. coli* concentration levels on carcasses after chilling proved to be a useful tool to reduce *Campylobacter* levels on postchill carcasses (Habib et al., 2012).

The aim of the study was to identify which processing steps contribute to increases or decreases in *Campylobacter* and *E. coli* concentrations in two slaughterhouses. Moreover the purpose was to identify whether the impact of the processing steps on bacterial contamination levels varied within and between slaughterhouses and whether the impact was similar for both *Campylobacter* and *E. coli*.

2. Materials and methods

2.1 . Slaughterhouses

The samples were taken in two commercial broiler chicken slaughterhouses. Slaughterhouse 1 is located in Germany and daily processes 130 000 broilers, whereas Slaughterhouse 2 is situated in The Netherlands and daily processes 240 000 broilers. The slaughterhouses were selected because of similarities in the processing equipment applied. During the study, the stunning, scalding and defeathering equipment was modernised in Slaughterhouse 1 prior to trials in 2013. The processing parameters remained the same.

2.2. Collection and preparation of samples

Thermotolerant *Campylobacter* and *E. coli* concentrations during broiler processing were measured in 21 trials. Eleven trials were performed in Slaughterhouse 1 (trials' ID: A, B, C, I, K, L, N, O, Q, R, U). Ten trials were performed in Slaughterhouse 2 (trials' ID: D, E, F, G, H, J, M, P, S, T). The trials were performed between June–October 2012 (trials A–M) and June–October 2013 (trials N–U). This sampling period was chosen in order to increase the probability of *Campylobacter* positive flocks (European Food Safety Authority, 2010c). Each trial was performed on a different day and included one batch, defined as a group of chickens raised together in one shed (European Food Safety Authority, 2011).

The *Campylobacter* status of the batch was ascertained by the slaughterhouses. In Slaughterhouse 1, bootswabs (in 2012 and 2013) and cloacal swabs (in 2012) at farms were taken 2–3 days prior to sampling in the slaughterhouse. The bootswabs were enriched in Campy Food broth (bioMérieux SA, Marcy l'Etoile, France), the cloacal swabs in Preston broth (prepared according to manufacturer guidelines — Oxoid). From the enrichment broth 1 ml was taken for further analysis and the positivity was checked by PCR with a detection limit of 100 CFU/ml. In Slaughterhouse 2, faecal droppings were collected at farms one week prior to sampling in the slaughterhouse. The faecal droppings were streaked on mCCDA, incubated and confirmed according to a Dutch national method (Anonymous, 2010). The limit of detection was 100 CFU/g.

During the trials, the first samples were collected after at least 1000 carcasses of the investigated batch had passed through the line, in order to avoid potential cross-contamination from the previously slaughtered batch. Samples were collected after the following processing steps: 1) just after bleeding, 2) just after scalding, 3) just after defeathering, 4) after evisceration and evisceration spraying cabinet but before inside and outside washing and 5) just after chilling. These steps were chosen, because the most dynamic changes in bacterial contamination levels were reported after these steps (Rosenquist et al., 2006).

The sampling plan to collect quantitative data is presented in Table 1. Whole carcass rinse was performed as described previously (Pacholewicz et al., 2013). The carcasses were removed from the line after selected processing steps. The cloacae of the non-eviscerated carcasses were plugged with a fibre tampon to prevent faecal and intestinal leakage while rinsing. Prevention of leakage of faecal material as a result of plugging was compared to results from rinsing the carcasses that were plugged and sealed (results not shown). Plugging and sealing of the vent were previously reported to prevent the faecal leakage (Berrang et al., 2001). After placing the carcasses into sterile plastic bags (Hevel, Zaandam, The Netherlands), 500 ml of peptone saline was added and the carcasses were shaken by hands for 60 s (Anonymous, 2000; Nauta et al., 2007; Reich et al., 2008). The same volume of the rinse was used for carcasses with feathers, defeathered or eviscerated carcasses to overcome differences in bacterial recovery, because removal rate was reported to differ with different volumes of rinse fluid (Williams et al., 2010).

In addition positivity of caeca from carcasses sampled after evisceration was checked in trials J–U. Caecal material was plated on Campy Food Agar and in case of no growth, enrichment was done according to the Dutch national method (Anonymous, 2010).

Breast skin samples after chilling were additionally collected during trials in 2013. The purpose of collecting the breast skin samples was to compare results from rinse and breast skin. The skin samples were collected as previously described (Anonymous, 2011). In short: 25 g (+/− 5 g) of skin from breast corpus was cut and placed in a stomacher bag.

Samples collected after different processing steps do not correspond to the same carcass, except for the caecal samples. These samples were collected from the same eviscerated carcasses that were sampled by the whole carcass rinse method.

The number of samples collected per trial for each sampling location was different (Table 1). In trials A–D — 3 samples were collected, in trials E–G — 4 and in trials H–U — 8. The difference in the number of