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Identification of risk factors for *Campylobacter* contamination levels on broiler carcasses during the slaughter process

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ABSTRACT

Campylobacter carcass contamination was quantified across the slaughter line during processing of *Campylobacter* positive batches. These quantitative data were combined together with information describing slaughterhouse and batch related characteristics in order to identify risk factors for *Campylobacter* contamination levels on broiler carcasses. The results revealed that *Campylobacter* counts are influenced by the contamination of incoming birds (both the initial external carcass contamination and the colonization level of caeca) and the duration of transport and holding time that can be linked with feed withdrawal period. In addition, technical aspects of the slaughter process such as a dump based unloading system, electrical stunning, lower scalding temperature, incorrect setting of plucking, vent cutter and evisceration machines were identified as risk factors associated with increased *Campylobacter* counts on processed carcasses. As such the study indicates possible improvements of the slaughter process that can result in better control of *Campylobacter* numbers under routine processing of *Campylobacter* positive batches without use of chemical or physical decontamination. Moreover, all investigated factors were existing variations of the routine processing practises and therefore proposed interventions are practically and economically achievable.

1. Introduction

Campylobacteriosis remains the most frequently reported zoonotic disease in humans in the European Union (EU) with confirmed number of cases exceeding 214,000 in 2013 (EFSA 2015). However, it is estimated that annually more than 9 million people in the EU can suffer from campylobacteriosis (Havelaar et al. 2012). Multiple sources for *Campylobacter* infection have been identified (Kittl et al. 2013; Lévesque et al. 2013), but 20 to 30% of campylobacteriosis cases in humans in the EU are thought to be attributed to the consumption and handling of broiler meat (EFSA 2011). There is a need for interventions in the broiler meat sector aiming to reduce human exposure to this pathogen.

At present, it has shown to be challenging to prevent or reduce *Campylobacter* colonization of broiler flocks at the end of the rearing period (Hermans et al. 2011; Lin 2009; Newell et al. 2011; Wagenaar et al., 2013). In addition, it has been reported that the main consumer risk is in particular associated with highly contaminated broiler meat rather than *Campylobacter* presence as such (Nauta et al. 2009). This implies that, in the short-term perspective, intervention measures reducing *Campylobacter* contamination levels during broiler slaughtering and further processing is considered more practical and effective.

Several interventions at the slaughterhouse level including freezing (Tustin et al. 2011), crust freezing (Haughton et al. 2012), ultraviolet light (Haughton et al. 2011), irradiation (Kudra et al. 2012) and chemical decontamination (Riedel et al. 2009) have been proposed or applied to lower *Campylobacter* counts on poultry meat. Nevertheless, consumer acceptability (MacRitchie et al., 2014) as well as economic (Havelaar et al. 2007) and legal (Hugas and Tsigarida 2008) arguments limit the implementation of these practises particularly in Europe. It is expected, that *Campylobacter* counts on broiler carcasses can be also reduced by optimization of the technical and hygiene-related factors (Habib et al. 2012; Wagenaar et al., 2013). To enable slaughterhouses to implement such an intervention, factors that influence *Campylobacter* carcass contamination and in particular the numbers of *Campylobacter* on carcasses derived from positive batches need to be identified. Therefore, a risk factor analyses was performed on *Campylobacter* data obtained across the

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slaughter line in six Belgian slaughterhouses during processing of *Campylobacter* positive broilers.

2. Material and methods

2.1. Selection of slaughterhouses

Six Belgian broiler slaughterhouses with a minimum annual production capacity of 10 million broilers each were included in the study and coded from A to F. Based on data obtained during the 2008 EFSA baseline study two of them (slaughterhouses B and E) had higher *Campylobacter* prevalence on chilled carcasses in comparison to slaughterhouses C and D (Habib et al. 2012). Slaughterhouses A and F were not included in the 2008 EFSA baseline study. Each slaughterhouse was approved by the national food safety agency according to the European hygiene legislation (EU 852/2004 and EU 853/2004) and is thus operating under government inspection. Additionally all slaughterhouses had a quality management system in place based on certified voluntary standards such as BRC and IFS. They were all equipped with an automated processing line, with slaughter capacities ranging between 6 and 12 thousand birds per hour.

2.2. Slaughterhouse and batch related process characteristics

To collect information about the technical aspects of the slaughter process, two questionnaires were designed, based on literature review. The questionnaires consisted of a series of questions related to factors potentially associated with *Campylobacter* counts on broiler carcasses. The first questionnaire was completed by researchers (authors TS, JB, LDZ) in a separate visit, before the first sampling took place. During this initial visit the slaughterhouse representative was interviewed and a slaughter line was checked by the researcher. This initial questionnaire focused on general management and technical slaughter line characteristics. Information obtained by this questionnaire resulted in the identification of 20 slaughterhouse related explanatory variables (Table 1). A second questionnaire focused on batch related processing conditions and was completed during every sampling visit based on researcher's (authors TS, JB) observations and check of the slaughterhouse records. Information obtained by this second questionnaire resulted in the identification of 9 batch related explanatory variables (Table 1).

2.3. Collection of batch related bacteriological data

From each batch six feather samples (*ca.* 10 g of feathers from each carcass collected after bleeding), six caecal and duodenal samples (*ca.* 1 g of caecal and duodenal content collected after the evisceration process) were analysed. Additionally, scalding water samples (*ca.* 100 ml) were collected from the beginning and the end of every scalding tank. Likewise, chilling water was also sampled in slaughterhouse C. If the sampled batch was not the first during the processing day, pooled caecal ($n = 10$) and duodenal ($n = 10$) samples were examined from the preceding slaughtered batch. For all sample types, *Campylobacter* was enumerated by direct plating on CampyFood Agar (CFA; bioMérieux, France), after an incubation time of 48 h at 41.5 °C under microaerobic conditions. If applicable, at least four presumptive positive colonies were confirmed by PCR assay (Vandamme et al. 1997). Obtained results are summarized in Table 1 as batch related bacteriological data and the mean values for each batch are presented in Table A (supplemental material).

2.4. Collection of Campylobacter quantitative carcass contamination data

Previously collected data (Seliwiorstow et al. 2015), describing *Campylobacter* carcass contamination along the processing line during slaughter of three *Campylobacter* positive batches (*i.e.* birds originated from one broiler house and slaughtered at the same day) in each of four slaughterhouses (A–D), were used in the present study. In the frame of the present study, two additional *Campylobacter* positive batches were sampled in slaughterhouses A, B and D, but not in slaughterhouse C which had stopped its production activities. Furthermore, two other slaughterhouses (E and F) were included in the present study and five batches were sampled in each of these. Altogether, 28 *Campylobacter* positive broiler batches were sampled throughout different steps in the slaughter line during the period February 2011 to November 2013.

Identification of *Campylobacter* positive batches on farms, the sampling strategy at the slaughterhouse and *Campylobacter* enumeration was performed as described previously (Seliwiorstow et al. 2015). Briefly, the *Campylobacter* status of each batch was defined by analysing caecal droppings collected on-farm using modified Cefaperazone Charcoal Desoxycholate Agar (mCCDA; Oxoid, England). After 24 h of incubation under microaerobic conditions at 41.5 °C, presumptive *Campylobacter* colonies were confirmed by Gram staining and microscopic observation. *Campylobacter* positive batches were sampled at the slaughterhouse level by collecting six breast skin samples (Baré et al. 2013) at seven sites along the slaughter line: after bleeding, after plucking, after evisceration, after crop puller, before and after final washing (*i.e.* before and after inside-outside washer), and after chilling. Practical limitations hampered the sampling after scalding which was therefore not included. In slaughterhouse C the final washing step (inside–outside washer) was replaced by short water chilling followed by air chilling. In this slaughterhouse, breast skin samples were taken before and after water chilling instead of before and after washing. For all collected samples, *Campylobacter* was enumerated by direct plating on CFA plates after an incubation time of 48 h at 41.5 °C under microaerobic conditions. If applicable, at least four presumptive positive colonies were confirmed by PCR assay (Vandamme et al. 1997).

2.5. Statistical analysis

For samples which were below the enumeration limit (10 cfu/g), counts were set to one-half of the enumeration threshold (Rosenquist et al. 2006). Mixed-effects negative binomial regressions (Anonymous, 2015), including batch as a random factor, were applied to investigate the associations between *Campylobacter* counts and the potential explanatory variables at each of five selected sampling sites (after bleeding, after plucking, after evisceration, after washing and after chilling). A stepwise forward inclusion model-building strategy was used, including at each step the significant variable until only the non-significant variables remained. If more than one explanatory variable was significant at a certain step of the model building process, Akaike information criterion (AIC) was applied to decide which variable should be included first in the model. Statistical analyses were carried out using commercial software (Stata/SE 13.1 StataCorp LP, College Station, TX) and a significance level of 5% was used.

3. Results and discussion

In total, *Campylobacter* was quantified on 1176 broiler carcasses originating from 28 *Campylobacter* positive batches sampled in six slaughterhouses along the slaughter line (supplemental material; Fig. A.1). *Campylobacter* counts collected at five sampling sites were included in the risk factor identification: i) after bleeding and ii) after chilling, as the beginning and the end of the slaughter process, respectively and additionally, iii) after plucking, iv) after evisceration and v) after washing as the latter three steps prior being identified as critical steps in the slaughter process leading to changes in *Campylobacter* numbers on broiler carcasses (Allen et al. 2007; Berrang and Dickens 2000; Cason et al., 1997; Izat et al. 1988; Pacholewicz et al. 2015; Reich et al. 2008; Rosenquist et al. 2006;