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Campylobacter carcass contamination throughout the slaughter process of *Campylobacter*-positive broiler batches

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ABSTRACT

Campylobacter contamination on broiler carcasses of *Campylobacter* colonized flocks was quantified at seven sampling sites throughout the slaughter process. For this purpose, in four slaughterhouses samples were collected from twelve *Campylobacter* positive batches.

Broilers from all visits carried high numbers of campylobacters in their caeca (≥7.9 $\log_{10}$ cfu/g). *Campylobacter* counts on feathers (up to 6.8 $\log_{10}$ cfu/g), positively associated with the breast skin contamination of incoming birds and carcasses after plucking, were identified as an additional source of carcass contamination. A high variability in *Campylobacter* carcass contamination on breast skin samples within batches and between batches in the same slaughterhouse and between slaughterhouses was observed. In slaughterhouses A, B, C and D *Campylobacter* counts exceeded a limit of 1000 cfu/g on 50%, 56%, 78% and 11% of carcasses after chilling, respectively. This finding indicates that certain slaughterhouses are able to better control *Campylobacter* contamination than others.

Overall, the present study focuses on the descriptive analysis of *Campylobacter* counts in different slaughterhouses, different batches within a slaughterhouse and within a batch at several sampling locations.

1. Introduction

Campylobacter is the most common cause of bacterial foodborne infection in humans in developed countries (WHO, 2012). According to the European Food Safety Authority report (EFSA, 2014), the notification rate of human campylobacteriosis in 2012 exceeded 50 per 100,000 of population in the European Union. Common clinical symptoms of campylobacteriosis are diarrhea, vomiting, abdominal pain and fever but infection can also lead to severe complications such as Guillain-Barré syndrome (Rees et al., 1995), reactive arthritis (Hannu et al., 2002) and also irritable bowel syndrome (Gradel et al., 2009). Campylobacteriosis in humans is mainly caused by two species: *Campylobacter jejuni* and *Campylobacter coli* (Park, 2002). Poultry are considered the main *Campylobacter* reservoir (Humphrey et al., 2007) and it is estimated that 50–80% of human campylobacteriosis cases may be attributed to the chicken reservoir as a whole, while 20–30% is assumed to be linked to handling, preparation and consumption of broiler meat (EFSA, 2011). Several risk assessment studies concluded that the reduction of *Campylobacter* numbers on carcasses would lead to a lower number of human cases associated with handling and consumption of broiler meat (Rosenquist et al., 2003; Nauta et al., 2005; Uyttendaele et al., 2006; Havelaar et al., 2007).

An effective way to protect public health from *Campylobacter* foodborne infections could be the decrease of the prevalence and numbers of *Campylobacter* in broiler chickens at the primary production stage (EFSA, 2010). However, at present, application of stringent general biosecurity interventions at farm level cannot prevent *Campylobacter* infection of broiler flocks at the end of the rearing period (Newell et al., 2011).

It is documented that contamination of carcasses' surface with *Campylobacter* occurs when *Campylobacter* positive flocks are slaughtered (Newell et al., 2001; Miwa et al., 2003) and it has been further proven that slaughter of birds with low mean *Campylobacter* colonization levels in their caeca results in lower carcass contamination and consequently in lower public health risk (Reich et al., 2008; Rosenquist et al., 2006). Since carcasses contaminated with high numbers of *Campylobacter* are related with high consumers' health risk (Callicott et al., 2008; Nauta and Havelaar, 2008), quantitative data are in the higher interest of public health than presence–absence testing.

According to EFSA, certain slaughterhouses can better control the *Campylobacter* counts on carcasses than others. This suggests that there is an opportunity for reducing *Campylobacter* numbers during the slaughter process (EFSA, 2011). Similarly, a recent study in Belgium showed significant differences in *Campylobacter* counts on carcasses after chilling between 9 slaughterhouses (Habib et al., 2012). Possibly,

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this variability might be explained by external birds' contamination, intestinal colonization level and by carcass contamination at the earlier slaughter stages.

Thus, the aim of the present study was to provide insights in *Campylobacter* counts on broiler carcasses throughout the slaughter process of *Campylobacter* positive flocks. The study was set-up to take into account the intravariability and intervariability in broiler slaughterhouses.

2. Materials and methods

2.1. Slaughterhouse profiles

Four Belgian slaughterhouses were selected based on data obtained during 2008 EFSA Baseline study. At that moment two of them, slaughterhouses A and C, were identified as slaughterhouses with both high *Campylobacter* prevalence (65% and 56%, respectively) and high percentage of carcasses contaminated with more than 3 $\log_{10}$ cfu/g (35% and 32%, respectively). On the other hand slaughterhouses G and H had lower prevalence of *Campylobacter* (42% and 36%, respectively) and also lower percentage of carcasses contaminated with more than 3 $\log_{10}$ cfu/g (14% and 19%, respectively; Habib et al., 2012). Selected characteristics for each chosen slaughterhouse are summarized in Table 1.

2.2. Identification of Campylobacter positive broiler flocks

Industrially reared flocks, namely a group of more than 12 thousand birds at 6 weeks of age reared in the same broiler house, were selected from the slaughter plan provided by the slaughterhouses. To identify *Campylobacter* positive broiler flocks, caecal droppings were collected at the farm three days before the scheduled slaughter day and analysed for the presence of *Campylobacter* by spreading collected caecal droppings on modified Cefaperazone Charcoal Desoxycholate Agar (mCCDA; *Campylobacter* blood free selective medium CM0739 plus selective supplement SR0155H [Oxoid, England]). Plates were incubated under microaerobic conditions at 41.5 °C for 24 h. Presumptive *Campylobacter* colonies were confirmed by Gram staining and microscopic observation. Birds from a *Campylobacter* positive broiler flock were further sampled during the slaughter process.

2.3. Sampling strategy at the slaughterhouse

In each of the four slaughterhouses, three batches (i.e. birds from one flock slaughtered at the same day) were sampled, resulting in 12 visits in the period from February to November 2011. During each visit six broiler carcasses were collected at each of the following sampling sites: after bleeding, after plucking, after evisceration, after crop puller, before and after washing (i.e. before and after final inside–outside washer), and after chilling (Table 2). Practical limitations hampered the sampling of carcasses after scalding. In slaughterhouse C, no final inside–outside washer was applied (Table 2). In this slaughterhouse, six carcass samples were taken before and after chilling tanks. Additionally, during every visit six intestinal packages were collected after the evisceration process (Table 2). The first sample at each sampling site was taken not earlier than 10 min after the beginning of the investigated batch. Further, sample collection was performed in a consecutive way over 1 h of slaughter. All samples were placed in sterile plastic bags, cooled on-site, transported to the laboratory under cooled conditions and analysed the same day.

2.4. Sample preparation and enumeration of Campylobacter

From carcasses after bleeding, *ca.* 10 g breast feathers were manually removed and analyzed separately. Next, from each carcass, *ca.* 10 g of breast skin was sampled for *Campylobacter* enumeration (Baré et al., 2013). From each intestinal package, one cecum and one duodenum was collected, immersed in ethanol and, after evaporation of the ethanol, approximately 1 g of content was pulled out into sterile plastic bags. All samples were homogenized with 0.1% peptone water (Bio-Rad Laboratories, Inc., Hercules, California, USA) at a ratio of 1:10, plated on CampyFood Agar (CFA; bioMérieux, France; Habib et al., 2011; Ugarte-Ruiz et al., 2012) and incubated under microaerobic conditions at 41.5 °C for 48 h. After incubation, colonies with typical *Campylobacter* morphology were counted and at least four of them per sample were confirmed by microscopic observation and by PCR (Vandamme et al., 1997).

2.5. Data analysis

Bacterial counts were $\log_{10}$-transformed to approximate the results to a normal distribution, which was further confirmed by a Shapiro–

Table 1
Selected slaughterhouses' characteristics.

	Slaughterhouse			
	A	B	C	D
Line speed[a]	11,000	12,700	9,000	12,000
Stunning	Electrical	Electrical	Electrical	Gas
Minimum scalding water temperature[b]	52.65 °c	52.30 °c	52.50 °c	54.70 °c
Maximum scalding water temperature[b]	53.70 °c	53.08 °c	53.70 °c	54.95 °c
Scalding time	150 s	250 s	138 s	145 s
Plucking time	35 s	42 s	43 s	42 s
Final inside-outside washer	Present	Present	Not present	Present
Water chilling tanks	Not present	Not present	Present	Not present
Water chilling temperature[c]	/	/	Tank 1 - 8.85 °C Tank 2 - 6.57 °C Tank 3 - 3.90 °C	/
Water sprays in chiller	Not present	Present	Present	Not present
Chilling time	105 min	120 min	135 min[d]	150 min
Air temperature in chiller	From 2.1 to 3 °C	From 3 to 5 °C	From 0 to 2 °C	From −2 to 0 °C
Carcasses temperature after chilling	From 2 to 4 °C	From 6 to 11 °C	From 5 to 6 °C	From 2 to 3 °C
Campylobacter positive samples[e]	56%	65%	42%	36%
Highly contaminated samples (≥3 $\log_{10}$ cfu/g)[e]	32%	35%	14%	19%

[a] Carcasses per hour.
[b] Average from the beginning and the end of each scalding tank measured once during each visit.
[c] Average from the beginning and the end of the chilling tank measured once during each visit.
[d] Including water chilling.
[e] Habib et al. (2012).