Quantification of the *Campylobacter* contamination on broiler carcasses during the slaughter of *Campylobacter* positive flocks in semi-industrialized slaughterhouses

Christian Vinuëza-Burgos, María Cevallos, Marco Cisneros, Inge Van Damme, Lieven De Zutter

**ABSTRACT**

*Campylobacter* contamination of broiler carcasses has been little studied in semi-industrialized slaughterhouses in developing countries, where several steps are carried out manually or with limited technology. In this study, we performed quantification of the *Campylobacter* contamination on carcasses at four steps in the slaughter process in three Ecuadorian slaughterhouses. Therefore, 15 *Campylobacter* positive batches were sampled in three commercial slaughterhouses. For every batch, caecal content and five samples of breast skin were taken and examined for *Campylobacter* counts at the following steps: after plucking, after evisceration, after final washing and after water chilling. Slaughterhouse C was the only slaughterhouse in which *Campylobacter* counts increased significantly after evisceration. No significant differences were found between counts after evisceration and after final washing (*P* > 0.05). In all slaughterhouses, a significant reduction of *Campylobacter* counts (0.11 to 2.55 log_{10} CFU/g) was found after the chilling step. The presence of chlorine in the chilling water was associated with the highest reduction in *Campylobacter* counts on the carcasses. A high variability of *Campylobacter* counts was found within and between batches slaughtered in the same slaughterhouse. *Campylobacter* counts in caecal content samples were not correlated with counts on carcasses after plucking nor after evisceration.

1. Introduction

Foodborne infections are of worldwide concern, especially in developing countries where the lack of epidemiological data and resources to control foodborne diseases needs to be addressed (Newell et al., 2010; WHO, 2015). Thermotolerant *Campylobacter* spp. are a major cause of foodborne gastrointestinal infections worldwide (WHO, 2015). Human campylobacteriosis is characterized by diarrhea, fever, abdominal cramps and vomiting and has been linked to the occurrence of Guillain-Barré syndrome, reactive arthritis and irritable bowel syndrome (Loshaj-Shala et al., 2015). The WHO (2015) estimated that *Campylobacter* caused 37,600 deaths per year globally. Furthermore, disability-adjusted life-years (DALYs) attributed to campylobacteriosis in developed countries is calculated to range from 1,568 in New Zealand to 2,850 in the USA (Skarp et al., 2015). It is estimated that in the European Union 50%–80% of campylobacteriosis cases may be attributed to the chicken reservoir as a whole while 20%–30% is linked to poultry meat consumption (EFSA, 2011; Skarp et al., 2015).

Additionally, if the infective dose of *Campylobacter* (=500 bacteria) is taken into account, the consumption of poultry meat contaminated with these bacteria may pose a public health concern (Nachamkin et al., 2008). *Campylobacter* loads on poultry meat are related to the level of contamination in processing plants (Pacholewicz et al., 2015a; Seliiwiorstow et al., 2015). Several studies reported that a reduction of *Campylobacter* counts on chicken carcasses leads to a risk reduction of campylobacteriosis cases associated with handling and consumption of chicken meat (Havelaar et al., 2007; Nauta et al., 2009; Uyttendaele et al., 2006). More specifically, production of batches of broiler carcasses with *Campylobacter* counts on neck and breast skin of maximal 500 to 1000 CFU/g may reduce the health risk by >50% (EFSA, 2011). To date, most of studies assessing *Campylobacter* contamination dynamics during slaughter have been performed under highly industrialized conditions. Therefore, there is a gap of knowledge about this latter issue in countries with emergent economies, where during industrialized slaughtering of broilers evisceration is carried out manually and in the chilling process water is used.

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Developing countries  
Slaughterhouse  
Quantification  
Chlorine  
Chilling water
2.1. Slaughterhouse profiles

Three poultry slaughterhouses, each belonging to a different integrated company, were included in this study. The characteristics of each slaughterhouse are listed in Table 1.

2.2. Identification of Campylobacter positive broiler flocks

Identification of Campylobacter positive flocks (birds reared in the same house) was performed 1 week before the chickens were slaughtered. Therefore, caecal droppings were collected in the broiler house at the farm and transported to the laboratory within 6 h. Direct plating of caecal droppings was performed 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 48 h. After incubation, colonies with typical Campylobacter morphology were counted and at least two colonies per sample were confirmed by microscopic observation.

2.3. Carcasses and caeca sampling during slaughter

In each of the three slaughterhouses, five batches originating from five Campylobacter positive flocks, were sampled, resulting in 15 visits in the period from July 2014 to April 2015. During each visit, five broiler carcasses were aseptically collected after each of the following slaughter steps: plucking, evisceration, final washing and water chilling. Additionally, one caecum from 25 chickens was collected. The first samples were collected 30 min after starting the slaughter process of the batch. Sample collection was performed in a consecutive way over 1.5 h of slaughter. All samples were placed in sterile plastic bags and transferred to a clean area in the slaughterhouse. There, approximately 10 g of breast skin was aseptically sampled for Campylobacter enumeration (Baré et al., 2013), placed in sterile plastic bags with filter (BagPage®, Interscience, Paris, France) and transported in an ice box to the laboratory within 2 h.

2.4. Sample preparation and enumeration of Campylobacter spp.

From each of the 25 caeca, the content was aseptically pooled. Therefore, all caeca were immersed in ethanol, and after evaporation of the ethanol approximately 1 g of content was collected in a sterile plastic bag. The pooled caecal content and the breast skin samples were homogenized in bacteriological peptone (Lab M, Lincashire, UK) at a ratio of 1:10, plated on Rapid Campylobacter Agar (Bio-Rad, California, USA) and incubated under microaerobic conditions at 41.5 °C for 48 h. After incubation, colonies with typical Campylobacter morphology were counted.

2.5. Data analysis

The detection limit of enumeration was 10 CFU/g for breast skin samples and 100 CFU/g for caecal samples. Quantification of breast skin samples that were below the enumeration limit was set to one-half of the enumeration threshold (Rosenquist et al., 2006). Campylobacter counts were log10-transformed prior to analysis.

Differences in Campylobacter counts were tested using random-effects generalized least squares regressions, including the batch as group variable. Differences in Campylobacter counts on caeces between the different steps (after plucking, after evisceration, after final washing and after chilling) were determined for each of the three slaughterhouses. Bonferroni corrections were applied for multiple testing.

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3. Results

During this study, 315 samples (15 caecal and 300 breast skin samples) were collected from 15 Campylobacter positive batches slaughtered in three slaughterhouses. Campylobacter counts in pooled caecal content samples varied considerably between batches (from 6.2 up to 11.1 log10 CFU/g; Table 2).

In order to get insight of the impact of the slaughter process on the Campylobacter contamination, quantification of Campylobacter was carried out after four processing steps.

The mean Campylobacter counts per sampling step in the three slaughterhouses is presented in Fig. 1. After plucking, mean counts in slaughterhouse C were significantly higher than in slaughterhouse A (P < 0.05). Afer evisceration, slaughterhouses B and C had significantly higher mean counts than slaughterhouse A (P < 0.001), while the difference between slaughterhouse B and slaughterhouse C was not significant (P > 0.05). After final washing, counts in

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Table 1

<table>
<thead>
<tr>
<th>Slaughterhouse</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Line speed (carcasses/h)</td>
<td>3000</td>
<td>3000</td>
<td>1000</td>
</tr>
<tr>
<td>Stunning</td>
<td>Electrical</td>
<td>Electrical</td>
<td>Electrical</td>
</tr>
<tr>
<td>Water temperature during scalding (°C)</td>
<td>56.9</td>
<td>52.9</td>
<td>64</td>
</tr>
<tr>
<td>Scalding time (s)</td>
<td>180</td>
<td>180</td>
<td>45</td>
</tr>
<tr>
<td>Plucking time (s)</td>
<td>180</td>
<td>180</td>
<td>45</td>
</tr>
<tr>
<td>Final inside-outside washer</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Water chilling tanks</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Temperature (°C) of the chilling water</td>
<td>Tank 1: 22</td>
<td>Tank 1: 25</td>
<td>Tank 1: 7</td>
</tr>
<tr>
<td>Free chlorine concentration in chilling water (ppm)</td>
<td>Tank 1: 0.5</td>
<td>Tank 1: 17</td>
<td>Tank 1: 0</td>
</tr>
<tr>
<td>Chilling time (min)</td>
<td>Tank 2: 0</td>
<td>Tank 2: 20</td>
<td>Tank 2: 0</td>
</tr>
<tr>
<td>Tank 3: 8</td>
<td>Tank 3: 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Addition of water in chilling tanks (l/carcass)</td>
<td>Tank 1: NA a</td>
<td>Tank 1: 1.5</td>
<td>Tank 1: 0</td>
</tr>
<tr>
<td>Tank 2: NA a</td>
<td>Tank 2: 1.5</td>
<td>Tank 2: 0</td>
<td></td>
</tr>
<tr>
<td>Tank 3: NA a</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Only potable water was used in slaughterhouse C.

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Table 2

<table>
<thead>
<tr>
<th>Batch number</th>
<th>Slaughterhouse</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>1</td>
<td>9.91</td>
</tr>
<tr>
<td>2</td>
<td>10.25</td>
</tr>
<tr>
<td>3</td>
<td>6.94</td>
</tr>
<tr>
<td>4</td>
<td>9.44</td>
</tr>
<tr>
<td>5</td>
<td>10.03</td>
</tr>
</tbody>
</table>
slaughterhouse C were significantly higher than in slaughterhouse A ($P < 0.05$). Finally, after chilling slaughterhouse C had higher counts than slaughterhouse A ($P < 0.05$) and slaughterhouse B ($P < 0.001$).

The mean counts per process step within each batch are shown for each slaughterhouse in Fig. 2. The mean Campylobacter counts and corresponding standard deviations within each step of the slaughter process per batch is given in the Supplementary Table.

Campylobacter counts on carcasses within a batch generally varied considerably at the 4 tested processing steps (standard deviations ranged between 0.11 and 1.12 log_{10} CFU/g). Also, the mean counts at the different slaughter steps for the 5 tested batches in each slaughterhouse showed a variation (Fig. 1).

After plucking, the mean Campylobacter count was 2.81 log_{10} CFU/g, 3.23 log_{10} CFU/g and 3.36 log_{10} CFU/g breast skin, while after water chilling the mean counts were 2.16 log_{10} CFU/g, 1.77 log_{10} CFU/g and 1.30 log_{10} CFU/g in slaughterhouse A and C, respectively. In slaughterhouse B, chilling led to a significant increase of the mean Campylobacter count by 1.59 log_{10} CFU/g (CI_{95%} [−1.86; −1.30]; $P < 0.001$).

There was no significant relation between caecal counts and Campylobacter counts on carcasses after all tested steps ($P > 0.05$).

### 4. Discussion

In this study, we present results of the Campylobacter quantification in 3 semi-industrialized slaughterhouses where hanging of live birds on the plucking line, rehanging of the carcasses on the evisceration line and evisceration are done manually. In order to evaluate the dynamics of Campylobacter counts in these slaughterhouses after plucking, after evisceration, after final washing and after chilling steps were selected as critical control points.

Campylobacter counts higher than 6 log_{10} CFU/g were found in pooled caecal content samples, which is consistent with values reported in other studies (Allen et al., 2007; Selwiwrostow et al., 2016; Stern et al., 2005).

After plucking the mean Campylobacter counts in the different slaughterhouses ranged from 2.81 up to 3.36 log_{10} CFU/g breast skin. These levels are similar to those obtained in other studies carried out in industrial slaughterhouses (Pacholewicz et al., 2015b; Selwiwrostow et al., 2015).

Although evisceration has been described as a critical step for Campylobacter contamination of carcasses (EFSA, 2011; Figueroa et al., 2009; Keener et al., 2004), it has also been described that an increase is not always observed in this step (Pacholewicz et al., 2015b; Rosenquist et al., 2006). In our study, only slaughterhouse C showed a small but significant increase of Campylobacter counts after evisceration. This observation could indicate that intestinal leakage caused by manual evisceration in these slaughterhouses has a minimal contribution to Campylobacter contamination.

Final washing of the carcasses showed no significant decrease in Campylobacter loads. A possible explanation could be related to the insufficient amount of water used and the application of water without disinfectants in this slaughter step (Bashor et al., 2004). Nevertheless, a lack of effect of final washing before chilling has also been observed even when chlorine is used in this washing (Berrang et al., 2007).

The reduction of Campylobacter counts after chilling in slaughterhouse C was less than in slaughterhouses A and B. In the former slaughterhouse no potable water was added during the chilling process, probably leading to a lower washing effect on the carcasses. In slaughterhouse A, a larger reduction of the mean Campylobacter count was observed. The reason for this reduction is unclear: the amount of potable water in chilling tanks (three liter/carcass) may have decreased probably leading to a lower washing effect. Chlorine compounds can reduce the number of Campylobacter on carcasses up to 2.9 log_{10} CFU/g (Berghaus et al., 2013; Berrang et al., 2007; Duffy et al., 2014). Besides, the washing effect of the addition of potable water in chilling tanks (three liter/carcass) may have decreased the amount of Campylobacter as shown elsewhere (Figueroa et al., 2009).

In the present study, intra-batch variation of Campylobacter counts...
Fig. 2. Campylobacter mean counts (log$_{10}$ CFU/g) on carcass breast skin collected at 4 steps of the slaughter process in slaughterhouses A, B and C. Significant differences of the mean count between 2 consecutive steps are indicated by an asterisk (* equals $P < 0.05$, ** equals $P < 0.001$).
was observed. This is in concordance with other studies showing similar results (Pacholewicz et al., 2015b; Selviiorstow et al., 2015). Concordantly, variability of Campylobacter counts among slaughterhouses within sampling steps was observed. This variation shows that some slaughterhouses are more able to control Campylobacter contamination levels during the slaughter of broilers than others, which has been described before (EFSA, 2010; Selviiorstow et al., 2015).

A positive correlation between Campylobacter counts in the ceca content of positive batches and counts on broiler carcasses has been reported (Berghaus et al., 2013). However, in this study such correlation was not observed. Factors such as the variable number of visceral rupture and leakage during evisceration have been mentioned to explain the lack of association of Campylobacter counts in feces and carcasses (Allen et al., 2007). Further research including other steps within the slaughter process should be done to understand this result.

Although the slaughterhouses studied are small in a global context, they are representative of such facilities in developing countries, where poultry is the main source of animal protein for human consumption. Data provided in this research may contribute to the understanding of the impact of the slaughter process on the level of Campylobacter contamination of broiler carcasses in developing countries. In order to tackle the Campylobacter contamination of broiler carcasses at slaughterhouse level, global measures should be taken. Increased biosecurity at farm level, adoption of rational interventions for contacts where improvements should be considered are final washing and addition of chlorine in chilling water Standardization of slaughtering procedures should also be reached. Moreover, this study could help national authorities and private companies to implement corrective measures at this kind of poultry slaughterhouses in order to bring safer poultry meat on the market.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijfoodmicro.2018.01.021.

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