International Journal of Food Microbiology 269 (2018) 75–79

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

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

Christian Vinueza-Burgos[a,*], María Cevallos[a], Marco Cisneros[a], Inge Van Damme[b], Lieven De Zutter[b]

[a] Facultad de Medicina Veterinaria y Zootecnia, Universidad Central del Ecuador, Quito, Ecuador
[b] Department of Veterinary Public Health and Food Safety, Faculty of Veterinary, Ghent University, Merelbeke, Belgium

ARTICLE INFO

Keywords:
Campylobacter
Developing countries
Slaughterhouse
Quantification
Chlorine
Chilling water

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 DALYs in New Zealand to 22.500 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; Seliwiorstow 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 accessing *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.

* Corresponding author at: Facultad de Medicina Veterinaria y Zootecnia, Universidad Central del Ecuador, Geronimo Leiton y Gato Sobral SN, Quito, Ecuador.
E-mail address: cvinueza@uce.edu.ec (C. Vinueza-Burgos).

https://doi.org/10.1016/j.ijfoodmicro.2018.01.021
Received 31 October 2017; Received in revised form 24 December 2017; Accepted 26 January 2018
Available online 31 January 2018
0168-1605/

Table 1
Characteristics of selected slaughterhouses.

Slaughterhouse	A	B	C
Line speed (carcasses/h)	3000	3000	1000
Stunning	Electrical	Electrical	Electrical
Evisceration	Manual	Manual	Manual
Water temperature during scalding (°C)	56,9	52,9	64
Scalding time (s)	180	180	45
Plucking time (s)	180	180	40
Final inside-outside washer	Present	Present	Present
Water chilling tanks	Present	Present	Present
Temperature (°C) of the chilling water	Tank 1: 22 Tank 2: 17 Tank 3: 8	Tank 1: 25 Tank 2: 3	Tank 1: 7 Tank 2: 3
Free chlorine concentration in chilling water (ppm)	Tank 1: 0.5 Tank 2: 0.5 Tank 3: 0	Tank 1: 17 Tank 2: 20	Tank 1: 0[a] Tank 2: 0[a]
Chilling time (min)	Tank 1: 10 Tank 2: 20 Tank 3: 45	Tank 1: 11 Tank 2: 60	Tank 1: 8 Tank 2: 50
Addition of water in chilling tanks (l/carcass)	Tank 1: NA[b] Tank 2: NA[b] Tank 3: NA[b]	Tank 1: 1.5 Tank 2: 1.5	Tank 1: 0 Tank 2: 0

[a] Only potable water was used in slaughterhouse C.
[b] NA: not available.

In this study, we aimed to provide insights in *Campylobacter* counts on carcasses at different steps during the slaughter of *Campylobacter* positive batches where manual evisceration and water chilling is applied.

2. Materials and methods

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 24 h. Presumptive *Campylobacter* colonies were confirmed by Gram staining and microscopic observation. Only *Campylobacter* positive flocks were sampled during the slaughter process.

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, Lancashire, 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 and at least two colonies per sample were confirmed by microscopic observation.

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 $\log_{10}$-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 carcasses 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. Differences between slaughterhouses were determined for each of the different steps separately. The relation between caecal content and the contamination level of carcasses was assessed using the mean caecal content counts of the batch as explanatory variable and carcass counts after plucking or counts after evisceration as response variable. A significance level of 5% was used. Statistical analyses were performed using STATA/IC 14.1 (StataCorp LP, TX, USA).

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 $\log_{10}$ 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 ($P < 0.05$). After 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

Table 2
Campylobacter counts ($\log_{10}$ CFU/g) in the caeca content of sampled batches.

Batch number	Slaughterhouse		
	A	B	C
1	9.91	7.51	11.12
2	10.25	10.48	10.11
3	6.94	6.20	10.09
4	9.44	7.64	10.21
5	10.03	9.59	10.20