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Usefulness of indicator bacteria as potential marker of *Campylobacter* contamination in broiler carcasses

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ABSTRACT

According to Regulation (EC) No 2017/1495, amending Regulation (EC) No 2073/2005, a slaughterhouse process hygiene criterion based on a limit of 10^3 CFU/g of *Campylobacter* for no more than 20 (for the years 2018 and 2019), 15 (for the years 2020–2024) and 10 (starting from 2025) of 50 neck skin samples of broiler carcasses could be an effective measure to reduce the incidence of human campylobacteriosis. In order to stimulate the poultry industry to improve the control of *Campylobacter* along the slaughter-line, the quantification of indicator bacteria such as *Escherichia coli* or *Enterobacteriaceae* could be a useful strategy. The aims of this study were: a) to investigate the possible relationship between *Campylobacter* and indicator bacteria counts at two different points of the broiler slaughter-line and b) to evaluate the probability that carcasses have *Campylobacter* counts above 10^3 CFU/g in relation to indicator bacteria counts. *E. coli*, *Enterobacteriaceae* and *Campylobacter* were simultaneously enumerated on neck skin samples of broiler carcasses sampled at the post-evisceration (n = 75) and at the post-chilling points (n = 75) of three Italian poultry slaughterhouses. In general, the log counts of all the investigated microorganisms were significantly lower at the post-chilling point, and the indicator bacteria (*E. coli* and *Enterobacteriaceae*) counts were significantly higher than *Campylobacter* counts at both sampling points. A multilevel linear mixed model, relating the *Campylobacter* $\log_{10}$ counts with the *E. coli* and *Enterobacteriaceae* $\log_{10}$ counts, showed that the *Campylobacter* $\log_{10}$ counts increased significantly for every additional *E. coli* $\log_{10}$ count, and this was more evident at the post-chilling than at the post-evisceration sampling point. With regards to the *Enterobacteriaceae*, the increase was similar at the two sampling points. An additional model, developed to assess the probability that carcasses would have *Campylobacter* counts above 3 $\log_{10}$ CFU/g, showed that this probability increased significantly if the level of *E. coli* count also increased. Carcasses classified as *Campylobacter* > 3 $\log_{10}$ CFU/g according to the observed results of *E. coli* ≥ 4 $\log_{10}$ CFU/g did have high *Campylobacter* counts. Conversely, it was not possible to conclude anything for carcasses with *E. coli* < 4 $\log_{10}$ CFU/g. These findings support the hypothesis that the monitoring of poultry carcasses for *E. coli* load could be useful to identify those heavily contaminated with *Campylobacter*, in order to implement control measures on the farms of origin of such batches, or improving the slaughter process of the plants where the heavily contaminated batches are found.

1. Introduction

Human campylobacteriosis is the most reported bacterial gastrointestinal disease in the European Union (EU), with around 246,307 cases notified in 2016 and among the cases with known hospitalization status, 28.5% were hospitalized (EFSA and ECDC, 2017). However, due to under-reporting, the real annual number of campylobacteriosis cases is estimated to be 9 million in the EU (Havelaar et al., 2009). According to the EFSA Scientific Opinion (EFSA, 2010b), handling, preparation and consumption of broiler meat may account for 20 to 30% of human cases, while 50 to 80% may be attributed to the poultry reservoir as a whole. Moreover, the EU baseline survey on the prevalence and counts of *Campylobacter* in broiler batches and carcasses carried out in 2008 showed considerable variations among Member States (EFSA, 2010a).

Risk assessment studies (Brynestad et al., 2008; Chapman et al., 2016; Lake et al., 2007; Lindqvist and Lindblad, 2008; Nauta et al., 2007; Rosenquist et al., 2003) and the EFSA opinion (EFSA, 2011) have focused on *Campylobacter* control options in broilers both on farm and

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at slaughterhouse level and estimated the impact of the different strategies on the reduction of the number of human cases. All studies concluded that the most effective measures should aim at reducing the prevalence of *Campylobacter* positive-flocks and the level of contamination of *Campylobacter* on broiler carcasses. In particular, strict implementation of biosecurity at primary production level and of GMP/HACCP during slaughtering is expected to reduce the level of *Campylobacter* colonization of broilers and finally the contamination level of carcasses and meat from colonized flocks (EFSA, 2011). In addition, risk assessment models clearly highlighted the relationship between *Campylobacter* counts on broiler carcasses and human campylobacteriosis cases and indicated that one or two $\log_{10}$ reductions in counts on chicken carcasses would significantly reduce the human health risk (Nauta et al., 2009).

The application of microbiological criteria/targets in the food chain is recognized as a useful tool to verify the processing hygiene in order to meet food safety objectives and obtain an appropriate level of protection (EFSA, 2010a; Jacxsens et al., 2009; Nauta et al., 2012; van Schothorst et al., 2009). The importance of, and urge for, such targets for *Campylobacter* is also supported by the EFSA opinion, estimating that at the European level, a public health risk reduction of *Campylobacter* infection from > 50% to > 90% could be achieved if all broiler batches sold as fresh meat complied with microbiological criteria based on a critical *Campylobacter* limit of 500 or 1000 CFU/g on breast and neck skin, respectively (EFSA, 2011).

Hence, the European Commission has recently published Regulation (EC) No 2017/1495, amending Regulation (EC) 2073/2005 and proposing a process hygiene criterion (PHC) for the poultry sector aiming at controlling contamination of carcasses during the slaughtering process. Under this legislation, neck skins from a minimum of 15 poultry carcasses shall be sampled at random after chilling and pooled to give 5 × 25 g final samples. Within a moving window of 50 samples, no more than 20 (for the years 2018 and 2019), 15 (for the years 2020–2024) and 10 (starting from 2025) may exceed the *Campylobacter* limit of 10^3 CFU/g. Moreover, this PHC could be used as a tool to classify slaughterhouses according to their capability to prevent or reduce microbiological hazards and as a tool to verify the hygiene management in slaughterhouses. However, indicator organisms, which are defined as a marker that reflects the general microbiological condition of a food or environment (Chapin et al., 2014), are better suited for use in process hygiene assessment compared to pathogenic microorganisms (EFSA, 2012). As suggested by EFSA in the opinion concerning the public health hazards to be covered by inspection of poultry meat, the evaluation of indicator organisms such as *E. coli* or *Enterobacteriaceae* on the carcasses can be useful to verify the hygiene management in slaughterhouses with the final aim of reducing the level of carcass contamination. Moreover, these indicator bacteria can be a valuable tool to classify poultry slaughterhouses according to their capability to prevent or reduce faecal contamination of broiler carcasses (EFSA, 2012).

Carcass contamination with intestinal contents inevitably occurs during slaughter. Poultry carcasses can become extensively contaminated due to the high levels of *Campylobacter* in faeces of animals entering the slaughterhouse or the characteristics of the slaughter process (Boysen et al., 2016; Seliwiorstow et al., 2015). The fact that faecal material is the source of contamination suggests that faecal bacteria such as *E. coli* or *Enterobacteriaceae* may be used as an indicator for *Campylobacter* contamination of broiler carcasses. The evaluation of the indicator bacteria counts of carcasses might have some practical merits. In fact, the enumeration of *E. coli* and *Enterobacteriaceae* is easy to perform, fast and cost-effective and it could allow the analysis of more samples than could be done if the same resources are allocated for *Campylobacter* enumeration.

Previous research pointed out the lack of available scientific data to support the correlation of indicators with pathogens in food products. If such correlation could be established, it could support the use of non-pathogenic organisms as indicators of process control with respect to pathogens (Saini et al., 2011), especially for the detection of situations which deserve particular attention to improve the *Campylobacter* status of poultry carcasses.

Therefore, the aims of the present study were multiple. The first objective was to investigate the possible relationship between *Campylobacter* and indicator bacteria (*E. coli* and *Enterobacteriaceae*) loads at two different points along the broiler slaughter-line. The decision to test the above-mentioned microorganisms at two sampling points was taken in order to study the variability of the counts between the two sampling points. In addition, the proposed microbiological criterion of 1000 CFU/g for *Campylobacter* at post-chilling was examined and the probability of having carcasses above this criterion in relation to a certain quantity of *E. coli* was evaluated. The decision to not compare *Enterobacteriaceae* with the proposed post-chilling *Campylobacter* criterion was due to the fact that within the *Enterobacteriaceae* group, *E. coli* is the most relevant microorganism in relation to faecal contamination of foods, and it is, thereby, the most widely-used indicator of faecal contamination (Smooth and Pierson, 1997). Thus, analyses comparing indicator microorganism levels with the proposed *Campylobacter* criterion were conducted utilizing *E. coli* only.

2. Materials and methods

2.1. Slaughterhouses and sampling

From June to September 2013, samples were collected in three Italian broiler slaughterhouses. Slaughterhouses were selected taking into account their potential capacity (size) and the available preliminary information on general technical characteristics in order to include abattoirs illustrative of the Italian broiler slaughterhouses. In particular, 1 large (> 10,000,000 broilers slaughtered per year) and 2 medium (between 1000,000 and 4,999,999 broilers slaughtered per year) sized slaughterhouses were included in the study. Information on the technical features such as stunning, scalding, plucking, evisceration and chilling of the three investigated slaughterhouses is reported in Table 1.

Each slaughterhouse was visited five times, and for each visit, one broiler batch was randomly selected for sampling. For the purposes of

Table 1
Technical information about the slaughterhouses.

Slaughterhouse ID	Stunning method	Scalding method and T (°C)	Plucking method	Evisceration method	Chilling method and T (°C)	Chilling time (minutes)
1	Electric water bath	Multi-bath counterflow (53 °C)	Vertical disk, counter-rotating disk and horizontal disk	Automatic drawing	Air tunnel (4; 0.5; 1)[a]	180
2	Electric water bath	Single-bath counterflow (48.5 °C)	Vertical disk, counter-rotating disk and horizontal disk	Automatic drawing completed by hands	Air tunnel (0; −5; −3)[a]	160
3	Electric water bath	Single-bath counterflow (52 °C)	Horizontal disk	Automatic drawing completed by hands	Air tunnel (0)[a]	55

[a] In these cases the chilling temperature is not homogeneous, thus the temperature in the starting, central and final parts of the tunnel are indicated.