International Journal of Food Microbiology 414 (2024) 110610

Contents lists available at ScienceDirect

International Journal of Food Microbiology

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

Status quo: Levels of *Campylobacter* spp. and hygiene indicators in German slaughterhouses for broiler and turkey

A. Beterams [a,1], C. Püning [b,1], B. Wyink [c], J. Grosse-Kleimann [d], G. Gölz [b], A. Schönknecht [b], T. Alter [b], F. Reich [a,*]

[a] German Federal Institute for Risk Assessment, Max-Dohrn-Strasse 8-10, D-10589 Berlin, Germany
[b] Institute of Food Safety and Food Hygiene, Center for Veterinary Public Health, Freie Universität Berlin, Königsweg 69, 14163 Berlin, Germany
[c] Frankenförder Forschungsgesellschaft, Potsdamer Straße 18a, 14943 Luckenwalde, Germany
[d] Department of Biometry, Epidemiology and Information Processing, University of Veterinary Medicine Hannover, Bünteweg 2, 30559 Hannover, Germany

ARTICLE INFO

Keywords:
E. coli
Total colony count
Process hygiene
Poultry

ABSTRACT

Poultry is a common reservoir for *Campylobacter* and a main source for human campylobacteriosis. With broiler being the predominant poultry for food production, most food safety related research is conducted for this species, for turkey, few studies are available. Although animals are typically colonized at the farm level, the slaughtering process is considered an important factor in re- and cross-contamination. We examined the development of *Campylobacter*, *E. coli* and total colony counts (TCC) after several processing steps in three broiler and one turkey slaughterhouses. Whole carcass rinsing and neck skin sampling was applied for broilers resulting in 486 samples in total, while 126 neck skin samples were collected for turkeys. A decrease in the loads of the different bacterial groups along the broiler slaughtering process was observed. *Campylobacter* mean counts dropped from 4.5 ± 1.7 $\log_{10}$ CFU/ml after killing to 1.6 ± 0.4 $\log_{10}$ CFU/ml after chilling. However, an increase in *Campylobacter* counts was evident after evisceration before the values again decreased by the final processing step. Although the *Campylobacter* prevalence in the turkey samples showed a similar development, the bacterial loads were much lower with 1.7 ± 0.3 $\log_{10}$ CFU/g after killing and 1.7 ± 0.4 $\log_{10}$ CFU/g after chilling compared to those of broilers. The loads of *E. coli* and total colony count of turkey were higher after killing, were reduced by scalding and remained stable until after chilling.

This study highlights trends during the slaughtering process in reducing the levels of *Campylobacter*, *E. coli*, and total colony counts for broiler and turkey carcasses, from the initial step to after chilling. These results contribute to our understanding of microbial dynamics during meat processing.

1. Introduction

The zoonotic pathogen *Campylobacter* is the major cause of foodborne bacterial gastroenteritis in the EU with approximately 250,000 reported infections in 2018 and approximately 128.000 cases in 2021 (EFSA, 2022). According to the European Food Safety Authority, the drop in infections could be a result of fewer reported infections probably due to the COVID-19 pandemic (EFSA, 2022). Infections with *Campylobacter* are mostly associated with the consumption of poultry meat, especially raw, undercooked and recontaminated meat or other commodities (EFSA, 2011). However, despite the fact that *Campylobacter* spp. are susceptible to several stressors, e.g., oxygen, osmotic stress and high temperature, they are still highly prevalent throughout the poultry production chain up to the fresh meat at retail (Alter and Scherer, 2006; Park, 2002), with an EU wide prevalence of 33 % in fresh broiler meat for the years 2017–2020 (EFSA, 2022).

Campylobacter spp. can colonize individual poultry in a short period of time resulting in high bacterial loads, e.g., in the caeca, and entire flocks become colonized quickly due to horizontal transmission (Stern, 2008; Szott et al., 2022). Birds from colonized flocks enter the slaughtering process with high *Campylobacter* counts on their feathers due to contact with dirt and fecal material in the poultry house as well as during transport (Rasschaert et al., 2020; Seliwiorstow et al., 2015).

Operation of poultry abattoirs comprises multiple processing steps,

* Corresponding author.
E-mail address: felix.reich@bfr.bund.de (F. Reich).
[1] Both authors contributed equally to this study.

https://doi.org/10.1016/j.ijfoodmicro.2024.110610
Received 17 August 2023; Received in revised form 22 December 2023; Accepted 29 January 2024
Available online 30 January 2024

starting with the arrival of the birds at the waiting area, followed by the stunning of the birds either in an electric water bath or with CO_2, followed by killing (Tondeur et al., 2019). After scalding and defeathering, the birds are re-hung in the evisceration line for further processing, ending with lung removal and inside outside-washing before the carcasses are chilled and finally cut (Tondeur et al., 2019). The processing steps in slaughterhouses can differ slightly (e.g. chilling systems, electrical stimulation after plucking), which could also influence the development of the bacterial contamination of the carcasses (Hauge et al., 2023). Most of the processing steps for different poultry species are similar. However, the time intervals of processing steps differ between broiler and turkey, due to the size difference between the species. While scalding can reduce the *Campylobacter* loads on carcasses (Hutchison et al., 2022), (re-)contamination can occur at subsequent processing steps due to contact with fecal material, whereas the most important processing steps for re-/cross-contamination of carcasses are defeathering and evisceration (Berrang et al., 2001; Guerin et al., 2010; Perez-Arnedo and Gonzalez-Fandos, 2019; Son et al., 2007).

The aim of this study was to gain further insight and up-to-date information on the impact of different processing steps on the distribution of *Campylobacter* spp. on broiler and turkey carcasses during the slaughtering process in Germany. To measure the general hygienic status and the influence of the different processing steps, the colony counts for *E. coli* and total colony counts (TCC) were investigated.

2. Material & methods

2.1. Sampling

Broiler samples were collected at three slaughterhouses (A, B, C) in Germany between September and November in 2021 and April and September 2022. Samples were taken at the three slaughterhouses on three individual visits, for a total of nine trials. The samples were taken from *Campylobacter*-positive flocks (with flock defined by Regulation (EC) No 2160/2003). To identify positive flocks, boot swabs were collected and tested via qualitative PCR analysis one week before slaughter by the companies, referring to Regulation (EC) No 200/2012. Seven processing steps were chosen: after killing, scalding, defeathering, evisceration, neck cutter/lung remover, inside/outside (I/O) washing and chilling. Sampling steps were chosen based on critical steps in the process that could influence contamination of the carcasses and cover the whole slaughtering process. In order to distribute the sampling over the entire processing time the sampling was performed during even time intervals. One carcass was collected at each processing step from after killing to after the inside/outside washer and this was repeated six times. Samples after chilling were collected at even time intervals as well. Neck skin samples were collected after neck cutter/lung remover and after chilling. Three neck skin samples were collected at each station and pooled according to Regulation (EC) 2073/2005. A total of 18 neck skin samples were pooled to form six pooled samples at each station per visit. Additionally, one caecal sample, pooled out of six intestines, was gathered to analyze the base contamination level of the flock.

Turkey samples were taken on three sampling days from one flock per day between May and July 2022 from one slaughterhouse (D), which processes only turkeys. No testing for *Campylobacter* positive flocks was conducted beforehand. The sampling started approximately two hours after the beginning of slaughtering and all samples were taken from the same flock. Neck skin was collected at the same processing steps as the broiler samples while the carcasses were still hanging on the line. On each sampling day six neck skin samples were taken per processing step and a further six caecal samples to examine the infection status of the flock were collected as well. The chilling period for turkeys was six to eight hours.

Broilers were sampled with whole carcass rinse (WCR) based on the assumption that not all parts of the carcass are contaminated equally (Corry et al., 2007); WCR is therefore a representative sample type to evaluate the contamination level of the surface of the carcass (Zhang et al. (2012). Broiler carcass samples were transferred into sterile plastic bags (400 × 500 mm, PA/PE 90, sealed bag, HEIFO GmbH & Co. KG, Osnabrück, Germany) with 400 ml rinsing solution (sodium chloride 0.85 %, peptone 0.1 %, 1.12535 (Merck, Darmstadt, Germany) + 0.75 g/l agar, LP0011 (OXOID, Wesel, Germany)), as described in ISO 17604:2015. The carcasses were rinsed inside and out for 60 s by turning the bag 180° every five seconds and moving it vigorously up and down six to ten times before turning again. By doing so, the rinsate covered the whole outer surface of the carcass as well as the body cavity. An aliquot of 200 ml of the rinse liquid was transferred to sterile plastic cups (300 ml, PP, VWR International GmbH, Darmstadt, Germany) for transportation. The rinse samples were cooled, transported to the lab, refrigerated and analyzed within 24 h. Neck skin was cut from additional carcasses by using a sterile knife and placing the sample into a plastic bag (broiler: Gefrierbeutel 1 l, 100 Stück, ja!, REWE, Köln, Germany; turkey: WhirlPak®, Madison, USA) according to DIN ISO 17604:2015. To achieve the 25 g weight per sample, three neck skin samples were combined for broiler. However, the turkey neck skin samples were processed individually. For both poultry, six sets of intestines were taken from the same flock and the caecum was used for analysis. For broiler from six intestines, 1 g of caecal material from each was pooled into one sample, while for turkey 25 g of the caeca samples were investigated individually. Samples were transported cooled to the laboratory and processed within 24 h.

2.2. Microbiological analysis

2.2.1. Qualitative detection of Campylobacter in turkey samples

To determine the presence of *Campylobacter* spp. qualitatively in turkey samples, 5 g of the respective neck skin samples were diluted 1:10 with Preston Broth (CM0689, OXOID) supplemented with 5 % horse blood (SR0050B, Thermo Fisher), *Campylobacter* selective supplement (SR0117, OXOID) and *Campylobacter* growth supplement (SR0232E, OXOID). The sample was blended and incubated at 37 °C, which was used due to logistical reasons, for 24 h under microaerobic conditions (generated with Anoxomat, Mart Microbiology B.V., Drachten, Netherlands). Approximately 10 µl of the enrichment culture were streaked onto modified charcoal cefoperazone deoxycolate agar (mCCDA, CM0739 (base) and SR0155 (supplement), both OXOID) and incubated at 37 °C for 48 h under microaerobic conditions. In the sampling process at the turkey slaughterhouse, quantifiable values were not always found for *Campylobacter* spp. at the different processing points.

2.2.2. Quantitative detection

At the laboratory, WCR samples from broilers were transferred to stomacher bags (432-008, VWR) and blended for 30 s to achieve a homogenous mixture. 25 g of the pooled neck skin from broiler or 25 g of individual neck skin from turkey were diluted 1:10 (w/w) with buffered peptone water (CM1049B, Merck, Darmstadt, Germany) and blended for 60 s (ISO 6887-1:2017). Pooled or individual caecal samples were homogenized by dilution with a 1:10 rinsing solution. For the microbiological analysis of the samples, tenfold dilution series were prepared.

In order to determine the *Campylobacter* load for broilers, 100 µl of the tenfold dilutions of WCR and neck skin were plated on mCCD agar and incubated at 41.5 °C for 48 h under microaerobic conditions according to ISO 10272-2:2017. For turkeys, one ml of the homogenized neck skin sample suspension was streaked on three mCCD agar plates. Furthermore, 50 µl of each dilution were streaked on mCCD agar with the drop-plating procedure, followed by an incubation at 37 °C for 48 h under microaerobic conditions. For both poultry, suspicious colonies were counted and up to three colonies per morphology were picked and streaked onto Mueller Hinton blood agar (CM0337, + sheep blood, OXOID) or Columbia blood agar (CM0331B + sheep blood, OXOID) followed by an incubation at 37 °C (turkey) /or 41.5 °C (broiler) for 48

h, in order to obtain fresh colonies for confirmation by microscopy for typical motility and morphology and oxidase tests (Oxidase strips for microbiology, SIGMA ALDRICH, Taufkirchen, Germany). The detection limit for the broiler WCR samples was 10 CFU/ml, i.e. 4000 CFU/carcass, 100 CFU/g for neck skin of broiler and 200 CFU/g for turkey.

To determine the bacterial load of *E. coli* for broiler samples, 1 ml of each 10-fold dilution was pipetted into petri dishes, poured over with trypton-bile-X-glucuronide agar (TBX, CM0945B, Merck) and incubated according to ISO 16649-3:2015. For turkey samples, 50 µl of the dilutions were streaked on TBX agar plates (CM0945, OXOID) with the drop plating procedure and then incubated at 44 °C for 18 h.

Total colony counts (TCC) were determined as follows for broilers: 1 ml of the 10-fold dilutions were pipetted into petri dishes and poured over with plate count (PC) agar (CM0325B, OXOID) and incubated at 30 °C for 72 h based on ISO 4833-2:2014; for turkey samples, the drop plating procedure was used to determine TCC by streaking 50 µl of each dilution onto PC agar (105,463, Merck).

Detection limits for *E. coli* and TCC were 1 CFU/ml for broiler WCR, 10 CFU/g for broiler neck skin, and 200 CFU/g for turkey.

2.3. Statistical analysis

The bacterial load data for broilers were $\log_{10}$ transformed to normalize their skewed distribution. To examine the reduction of the bacterial loads between the different processing steps and the three slaughterhouses, analyses of variance and, as post hoc-tests, multiple comparisons of the mean with Tukey-Kramer correction were conducted. Normality of distribution was assessed via Shapiro-Wilk tests. The statistical comparison of colony counts from WCR and neck skin samples was made via Student's *t*-test with a level of significance of 0.05. Furthermore, Spearman rank correlation coefficient was computed to examine the association between the three bacterial groups. All statistical analyses were made with SAS®, version 9.4, SAS Institute Inc., Cary, USA.

For statistical analysis of the turkey data, the bacterial loads were $\log_{10}$ transformed and GraphPad Prism (Version 5) was used. The presence or absence of normality distribution was tested with the D'Agostino-Pearson omnibus K2 normality test. The results of the qualitative analysis were compared to the results of the previous sampling point by using Fisher's exact test and a confidence interval of 95 %. To compare the *Campylobacter* spp. quantitative results at the different sampling points with each other, statistical analysis was performed by using Kruskal-Wallis One-Way ANOVA with Dunn's post-test along with a confidence interval of 95 %. A putative relationship among bacterial loads was analyzed by using a two-tailed, nonparametric Spearman correlation with a confidence interval of 95 %.

3. Results & discussion

3.1. Broiler

3.1.1. Development of bacterial loads during the slaughter process

In the three different slaughterhouses, the load of *Campylobacter* spp. in the WCR samples showed a similar change during the slaughtering process with an overall reduction until the end of the slaughter process (Fig. 1). For all slaughterhouses, the *Campylobacter* contamination levels of broiler carcasses were similar and within a range of 1 $\log_{10}$ CFU/ml at the individual processing step except for the first sampling point after killing, with a range of 2 $\log_{10}$ CFU/ml.

The contamination of broilers with *Campylobacter* decreased significantly to an average per slaughterhouse of 1.0–1.7 $\log_{10}$ CFU/ml after chilling, compared to the initial mean values of 3.9–5.3 $\log_{10}$ CFU/ml observed at the beginning of processing after killing. A notable drop in *Campylobacter* counts following scalding was observed, but there was a subsequent increase until after evisceration, before the values finally decreased by the final processing step. This overall trend is consistent with the findings reported by Marmion et al. (2021) in their study on broilers.

In a study conducted by Hutchison et al. (2022), the presence of *Campylobacter* contamination in neck skin samples from 22 broiler processing lines across 19 UK slaughterhouses was investigated. Their research examined similar processing steps as in the current study, revealing that *Campylobacter* levels on broiler carcasses did not exhibit a significant reduction until the end of the slaughtering process, even though significant reductions could be found in some slaughter lines or processing steps. In contrast, the current study found a consistent and significant decreasing trend throughout the slaughtering process in all studied slaughterhouses. In a study at Norwegian broiler slaughterhouses, Hauge et al. (2023) analyzed four sampling points (before scalding, after plucking, after evisceration, after chilling) and found a similar decreasing trend for TCC, *E. coli*, and *Campylobacter* with 2.1, 1.1 and 1.0 $\log_{10}$ CFU/g, respectively, by analyzing broiler neck-skin samples over the whole slaughtering process.

Besides the general trend, scalding was found to have had a strong impact on *Campylobacter* concentration of carcasses in this study. In all slaughterhouses *Campylobacter* were reduced from after killing with an average of 3.9–5.3 $\log_{10}$ CFU/ml to an average of 2.0–2.6 $\log_{10}$ CFU/ml after scalding. Similar reductions of *Campylobacter* with 1.2 and 1.6 $\log_{10}$ CFU/ml, respectively, were reported by Pacholewicz et al. (2015b) for two broiler slaughterhouses. A meta-analysis by Guerin et al. (2010) also showed reductions in the prevalence of *Campylobacter* for broiler chicken carcasses after scalding by 20–40 %. Despite the positive impact of scalding on the prevalence and bacterial load of *Campylobacter* (Alter

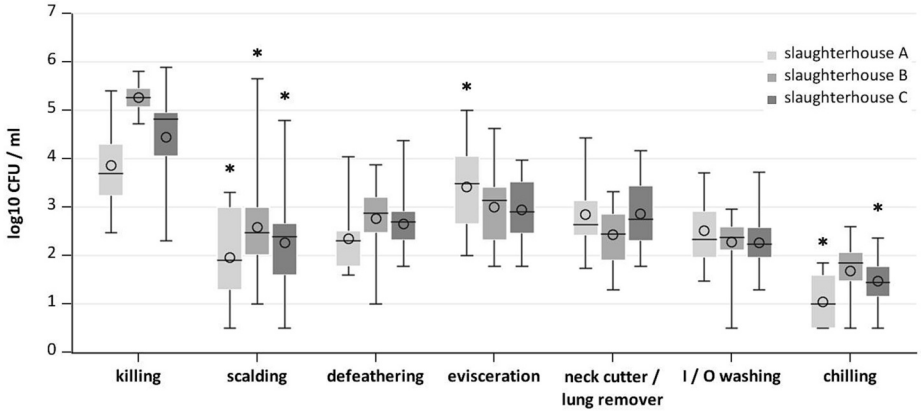

Fig. 1. *Campylobacter* spp. levels from broiler carcasses (WCR samples) after different stages of the slaughtering process for three broiler slaughterhouses (A, B, C). Shown are box plots as log10 CFU/ml with the median (line), mean (○), interquartile range and 95 % confidence interval ($n = 18$). Processing steps marked with * showed a significant difference to the previous sampling point with $p < 0.05$.

et al., 2005) due to the sensitivity of thermophiliic *Campylobacter* to high temperatures above the optimum growth temperature of 41 °C (Kim et al., 2021), this processing step can still be a reservoir for the pathogen, presenting the possibility for cross-contamination between different slaughter lots (Reich et al., 2008). However, the quantitative impact of recontamination due to scalding on *Campylobacter* levels would be expected to be rather low in *Campylobacter* positive flocks based on the observations of this study, because the initial load on birds entering the slaughter process was considerably higher.

There was a trend for an increase of the *Campylobacter* spp. loads from after scalding until after evisceration from averages of 2.0–2.6 $\log_{10}$ CFU/ml to 2.9–3.4 $\log_{10}$ CFU/ml, respectively. Defeathering and evisceration are critical steps in the production process where carcasses come into contact with fecal material through contaminated equipment or other carcasses, making them susceptible to *Campylobacter* contamination (Berrang et al., 2001; Rosenquist et al., 2006). In this study's three broiler slaughterhouses, *Campylobacter* contamination increased after defeathering and continued to rise after evisceration, consistent with findings by Hauge et al. (2023), Buess et al. (2019) and Pacholewicz et al. (2015b), whereas Hutchison et al. (2022) described the significant increase of *Campylobacter* already after defeathering, and evisceration had no additional effect. An increase of the bacterial load of broiler carcasses after defeathering and evisceration could be explained by the mechanical treatment of the carcasses, which can result in leakage of faeces and therefore in contamination of carcasses (Berrang et al., 2001; Musgrove et al., 1997; Pacholewicz et al., 2016; Yusufu et al., 1983). Another explanation by Zhang et al. (2020) states that the follicular cavities are filled with moisture, feather fragments, and debris and therefore may cause bacterial cross-contamination on the carcasses due to the contents of the follicular lumina, which can open during plucking. Seliwiorstow et al. (2015) suggested that the initial *Campylobacter* load influences the effects of defeathering and evisceration, possibly explaining conflicting findings such as no change (Berrang and Dickens, 2000) or a decrease (Allen et al., 2007) in *Campylobacter* counts. The different initial loads of *Campylobacter* in the three slaughterhouses in the current study led to no significant differences in the counts after the evisceration, which still highlights this processing step as a crucial point of the slaughtering process for *Campylobacter* contamination.

No significant change in the mean values of *Campylobacter* was found for broilers when comparing the sampling points evisceration and lung remover/neck cutter, similar to what was described by Hutchison et al. (2022). By the end of slaughtering, slaughterhouses A and C showed a significant decrease of *Campylobacter* spp. from on average 2.5 and 2.3 $\log_{10}$ CFU/ml, respectively after I/O washing to 1.1 and 1.5 $\log_{10}$ CFU/ml, respectively after chilling. Inside-outside (I/O) washing is considered an important step in poultry processing, but has received less attention in previous studies, as noted by Dogan et al. (2022). However, some studies, such as those conducted by Perez-Arnedo and Gonzalez-Fandos (2019) and Guerin et al. (2010), have focused on qualitative microbiological analysis of I/O washing, as did a study by Northcutt et al. (2005), where it was combined with the application of additives to the water source in U.S. broiler slaughterhouses (2005). For quantitative analysis for instance, Berrang and Bailey (2009) reported a decrease in *Campylobacter* counts of broilers after the I/O washer, with average reductions ranging between 1.3 ± 0.6 and 1.9 ± 0.8 CFU/g. Hutchison et al. (2022) reported mean reductions of *Campylobacter* of 0.9 log CFU/g in some slaughter lines after the I/O washer, consistent with the findings of the current study.

Application of temperatures below the growth optimum of bacteria, as done during chilling, can help to improve carcass hygiene by suppressing bacterial propagation and thereby improving shelf life. *Campylobacter* is known to be sensitive to temperature and drying and therefore chilling can lead to stagnation of cell growth or inactivation (Kim et al., 2021; Lindblad et al., 2006). Similar reductions have been reported in other studies, such as those conducted by Rosenquist et al. (2006), Guerin et al. (2010), and Duffy et al. (2014). The different results between slaughterhouses can be attributed to various factors. Firstly, the use of different chilling systems in slaughterhouses may influence the survival of *Campylobacter* on carcass surfaces, such as drying out over time when using air chilling, as described by Lindblad et al. (2006). On the other hand, pre-chilling with water tanks, as described by Blevins et al. (2020), could increase *Campylobacter* counts by creating a reservoir in the tanks. Another factor could be the survival of *Campylobacter* within feather follicles and skin crevices, where they can find a suitable microenvironment and water, making it difficult to remove bacteria during processing, as discussed by Chantarapanont et al. (2003).

The counts of *E. coli* decreased from around 4.5 $\log_{10}$ CFU/ml after killing to a mean of approximately 2 $\log_{10}$ CFU/ml over the whole process (Fig. 2). In slaughterhouse C, significant reductions were found between the first three processing steps, whereby the values decreased from 4.8 to 2.7 $\log_{10}$ CFU/ml between the sampling points after killing and after defeathering. However, an increase to 3.5 $\log_{10}$ CFU/ml was found after evisceration followed by a reduction from 3.0 $\log_{10}$ CFU/ml after the I/O washer to 1.8 $\log_{10}$ CFU/ml after chilling. Comparing the results from the samples after evisceration to those after the lung remover in slaughterhouse B, a decrease of *E. coli* was observed from 3.5 $\log_{10}$ to 2.5 $\log_{10}$ CFU/ml. A further reduction was seen between the last two sampling steps, after I/O washing and after chilling, with 2.9 $\log_{10}$ CFU/ml and 2.0 $\log_{10}$ CFU/ml, respectively.

A highly significant reduction ($p < 0.0001$) of the TCC was seen for all slaughterhouses over the whole process (Fig. 3). A decrease in TCC loads from after killing to after defeathering was observed, with colony counts dropping from 6.6 to 7.8 $\log_{10}$ CFU/ml to 5.0–5.9 $\log_{10}$ CFU/ml. A significant decrease of TCC was found in slaughterhouses B and C with CFUs ranging from 6.6 to 7.8 $\log_{10}$ CFU/ml after killing and 6.3–6.6 $\log_{10}$ CFU/ml after scalding. Slaughterhouse A and C showed significant reductions in TCC when comparing the results after I/O washing with 4.6–5.4 $\log_{10}$ CFU/ml and after chilling with 4.1–4.7 $\log_{10}$ CFU/ml.

The values for *E. coli* and TCC as indicator bacteria for hygiene in the slaughtering process of broilers decreased significantly over the process. Hauge et al. (2023) analyzed two broiler slaughterhouses in Norway using neck skin samples and described a similar development over the slaughtering process for TCC and *E. coli*, as did Buess et al. (2019), who examined neck skin samples in Swiss broiler abattoirs. After defeathering, *E. coli* and TCC loads decreased, while *Campylobacter* loads increased. This could be a result of the feather removal and their inhabitant bacteria. While most of the *Campylobacter* cells were already inactivated during scalding, the increase after defeathering could be due to mechanical treatment of the carcasses and fecal recontamination (Nauta et al., 2005). Studies on the effect of chilling on microbiological loads in broiler processing report diverse results. When water chilling was used, reductions in bacterial load were reported (Duffy et al., 2014) or partial increases in contamination of the carcasses were described (Elvers et al., 2011). The increases could be explained by cross-contamination in the immersion tanks (Sánchez et al., 2002), while others described a decrease in the bacterial loads when air chilling was used (Pacholewicz et al., 2015a). Overall, it was shown that all bacteria decreased until the end of the slaughter process, but the variation of *E. coli* and *Campylobacter* and the different reactions to various stressors need to be taken into account, when comparing them.

3.2. Turkey

3.2.1. Qualitative detection of Campylobacter

The prevalence of *Campylobacter* spp. along the production line was determined in the present study using a qualitative approach, with enrichment of 5 g neck skin sample (Fig. 4). While at the beginning of the slaughtering process 50 % (9/18) of the samples were positive for *Campylobacter* spp., the prevalence declined to 11 % (2/18) after scalding. A similar shift in the prevalence for turkey carcasses was

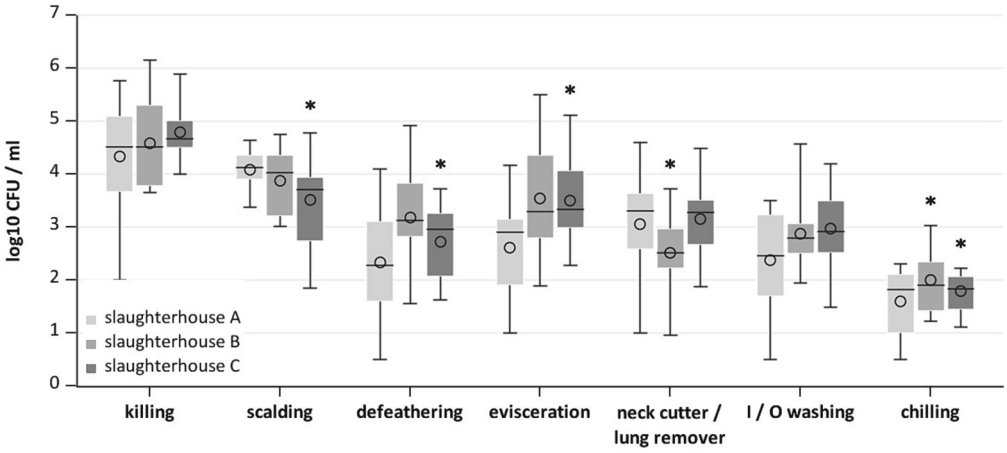

Fig. 2. *E. coli* levels from broiler carcasses (WCR samples) after different steps of the slaughtering process for three broiler slaughterhouses (A, B, C). Shown are box plots as log10 CFU/ml with the median (line), mean (○), interquartile range and 95 % confidence interval (n = 18). Processing steps marked with * showed a significant difference to the previous sampling point with $p < 0.05$.

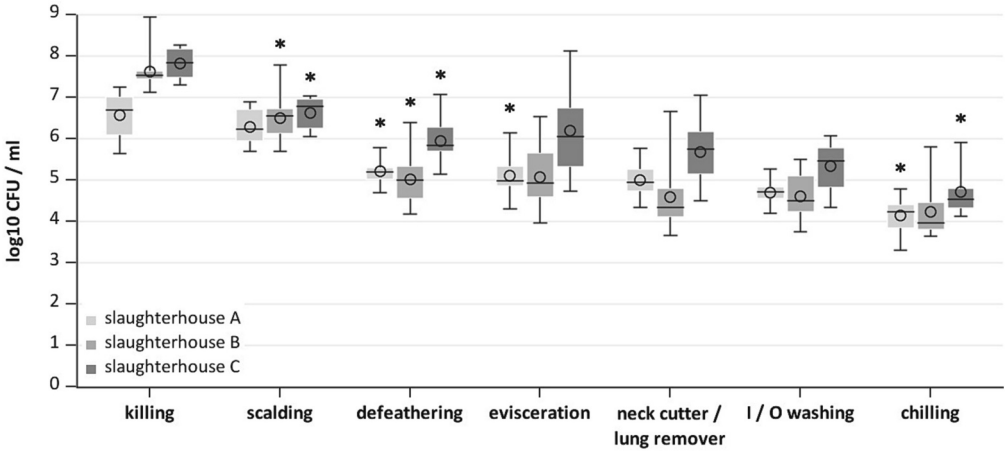

Fig. 3. TCC levels from broiler carcasses (WCR samples) after different steps of the slaughtering process for three broiler slaughterhouses (A, B, C). Shown are box plots as log10 CFU/ml with the median (line), mean (○), interquartile range and 95 % confidence interval (n = 18). Processing steps marked with * showed a significant difference to the previous sampling point with $p < 0.05$.

described by Alter et al. (2005), even though the *Campylobacter* prevalence was slightly higher after killing and scalding with 77 % and 37 %, respectively. Subsequently, a prevalence of 44 % (8/18) was determined after defeathering, which increased to 78 % (14/18) after evisceration and declined to 61 % (11/18) after chilling of the carcasses. The current data, where samples were chilled for six to eight hours, aligns with the results of Alter et al. (2005), where the prevalence of *Campylobacter* dropped from 72 % after evisceration to 67 % after chilling for 20 min, and further to 25.6 % after 24 h of chilling.

Overall *Campylobacter* spp. were detected in 89 % (16/18) of the turkey caecal samples, enriched from 1 g of caecal material. This suggests that the investigated turkey flocks were colonized with *Campylobacter*.

3.2.2. Quantitative detection

The mean values of the bacterial load, calculated using only *Campylobacter* spp. positive samples, ranged from 3.2 $\log_{10}$ CFU/g after defeathering to 1.7 $\log_{10}$ CFU/g after chilling of the carcasses (Table 1). After killing, 1.7 $\log_{10}$ CFU/g *Campylobacter* were found which were reduced to below 1 $\log_{10}$ CFU/g after scalding. While the bacterial load after defeathering was 3.2 $\log_{10}$ CFU/g, only one *Campylobacter* positive sample was found, which could explain the much lower CFU after evisceration of 1.8 $\log_{10}$ CFU/g. Following the neck cutter another increase of the bacterial load up to 2.6 $\log_{10}$ CFU/g was found. The bacterial load decreased to 2.3 $\log_{10}$ CFU/g after washing and finally to 1.7 $\log_{10}$ CFU/g after chilling. The latter value was slightly lower than the results from Atanassova et al. (2007), who investigated the bacterial loads of cooled turkey meat parts from a slaughterhouse with *Campylobacter* loads ranging from 1.9 to 2.3 $\log_{10}$ CFU/g.

There were no statistically significant differences in the *Campylobacter* loads of the neck skin samples at the different processing steps.

The bacterial loads of *Campylobacter* spp. on neck skin were significantly lower than those of *E. coli* or TCC (Fig. 5). However, over the course of the production line all bacterial loads showed similarities with a downward trend after scalding and an upward trend after defeathering. At the beginning of the slaughtering process the mean loads for *E. coli* and TCC were highest at 4.0 and 6.3 $\log_{10}$ CFU/g, respectively. After scalding, neither *Campylobacter* spp. nor *E. coli* were detected, while the TCC decreased by 2 $\log_{10}$ CFU/g on average. Following the further steps along the production line, the bacterial loads for *E. coli* and *Campylobacter* spp. showed upward trends, whereas the TCC loads remained stable. The bacterial loads for *Campylobacter* increased after the neck cutter, whereas the TCC showed a decrease and the *E. coli* level remained stable. Taking into account that only six out of 18 turkey samples tested positive for *Campylobacter*, we speculate the increase in the bacterial loads after the lung remover/neck cutter to be an outlier.

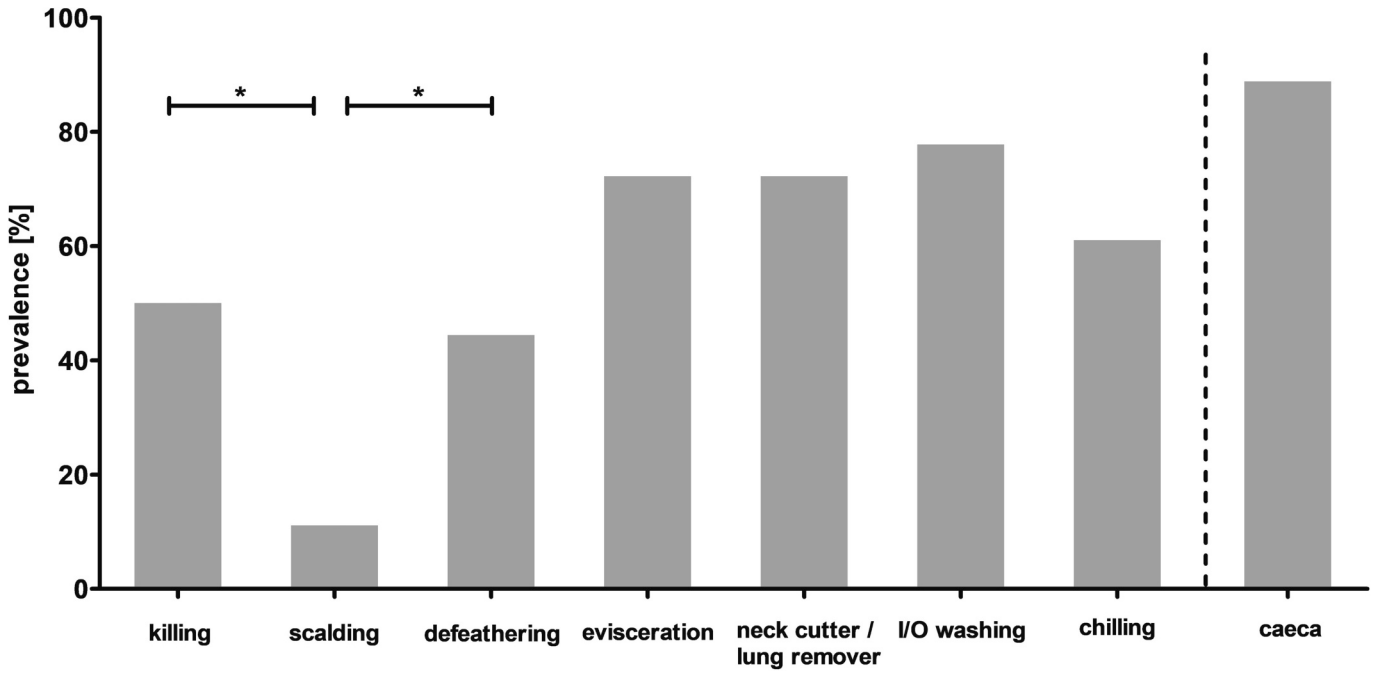

Fig. 4. Qualitative detection of *Campylobacter* spp. along the turkey production line in neck skin and in caeca samples. Shown is the prevalence of 18 samples per processing step, in percent. Processing steps marked with * showed a significant difference to the previous sampling point with $p < 0.05$.

Table 1
Bacterial loads of turkey neck skin samples for *Campylobacter* spp. at different steps of the slaughtering process. For calculation of the bacterial load, only *Campylobacter* spp. positive samples were included; N/A = not available.

Sampling point	Positive samples[a]/ analyzed samples (positive %)	25 % percentile (log_{10} CFU/g)	Mean (log_{10} CFU/g)	75 % percentile (log_{10} CFU/g)
Killing	6/ 18 (33 %)	1.4	1.7	2.0
Scalding	0/ 18 (0.0 %)	<1	<1	<1
Defeathering	1/ 18 (6 %)	N/A	3.2	N/A
Evisceration	5/ 18 (28 %)	1.4	1.8	2.2
Neck cutter	6/ 18 (33 %)	1.8	2.6	3.1
I/O washing	5/ 18 (28 %)	1.5	2.3	3.1
Chilling	4/ 18 (22 %)	1.4	1.7	2.1
Caeca	16/18 (89 %)	5.8	6.5	7.2

[a] Results of quantitative analysis.

While the mean loads of *E. coli* and TCC were still stable after the chilling of the carcasses, the loads of *Campylobacter* spp. showed a downward trend. Overall, the changes in the three bacterial groups along the turkey processing line were similar to those seen for broilers. However, comparison of the data of the *Campylobacter* loads between the two poultry species in this study is limited due to differences in the incubation temperature during the analysis of samples of broiler (41.5 °C) and turkey (37 °C).

3.3. Comparison of WCR and neck skin samples for broiler

The WCR and neck skin samples at the two steps after neck cutter/ lung remover and after chilling were compared (Figs. 6 and 7).

The neck skin sample is commonly used as the sample of choice within monitoring programs in slaughterhouses for both broiler and turkey in the EU, as a process hygiene criterium after chilling. Sampling after the lung remover was chosen as a step close to evisceration and with completed removal of neck and crop, to identify possible influences of the previous processing steps compared to after chilling. Various studies examined the use of the whole carcass rinse (WCR) as an alternative to the neck skin sampling (Nagel Gravning et al., 2021, Zhang et al., 2012, Gill and Badoni, 2005). In the current study, the mean bacterial concentrations of neck skin samples were comparable to the mean WCR bacterial concentrations at the first sampling station after lung remover ($p > 0.05$), while they were distinctly lower for WCR at the second station after chilling ($p < 0.0001$). The mean difference for *Campylobacter* was 1.0 log_{10} CFU/ml or g, for *E. coli* 0.5 log_{10} CFU/ml or g and 0.6 log_{10} CFU/ml or g for TCC (Table 2). Zhang et al. (2012) compared the WCR (300 ml sterile peptone water, 3 min shaking) and neck skin sample for TCC, *Pseudomonas* spp., lactic acid bacteria and *B. thermosphacta*. No difference was found between samples for TCC, wheras higher loads were found in the rinse compared to the excision samples for the other bacteria. In our study, the *Campylobacter* mean value of the neck skin samples after the lung remover was 2.9 log_{10} CFU/ g and 2.7 log_{10} CFU/ml for the WCR. After chilling, the values for neck skin were 1 log_{10} higher than those for the whole carcass rinse. Nagel Gravning et al. (2021) reported that the WCR, when carcasses were shaken for 30 s with 200 ml of sterile peptone water, captured the total bacterial load of naturally contaminated carcasses more efficiently than the neck skin sample, but still recovered 80–100 % of the WCR. The discrepancy between the two sampling methods after chilling can probably be explained by the drying of the carcass surface during chilling. The WCR could have disadvantages with dry carcasses, by removing fewer bacteria with rinsing compared to wet carcass surfaces at the other processing steps (Hutchison et al., 2022).

3.4. Correlation of the bacterial groups for broiler slaughterhouses

The correlation analysis can be used to determine whether there is a relationship between the quantitative change in bacteria and whether the hygiene indicators could be suitable for predicting *Campylobacter* contamination of carcasses. For the broiler slaughterhouses, medium correlations for WCR between *Campylobacter* and *E. coli* were found with $r = 0.45$ ($p < 0.0001$). A slightly stronger effect was observed when comparing the results from TCC and the *Campylobacter* spp. load, with $r = 0.52$ ($p < 0.0001$). The correlation was higher when comparing the *E. coli* counts with the values of the TCC with $r = 0.73$ ($p < 0.0001$). This correlation is not sufficient to reliably use *E. coli* and TCC as a hygiene

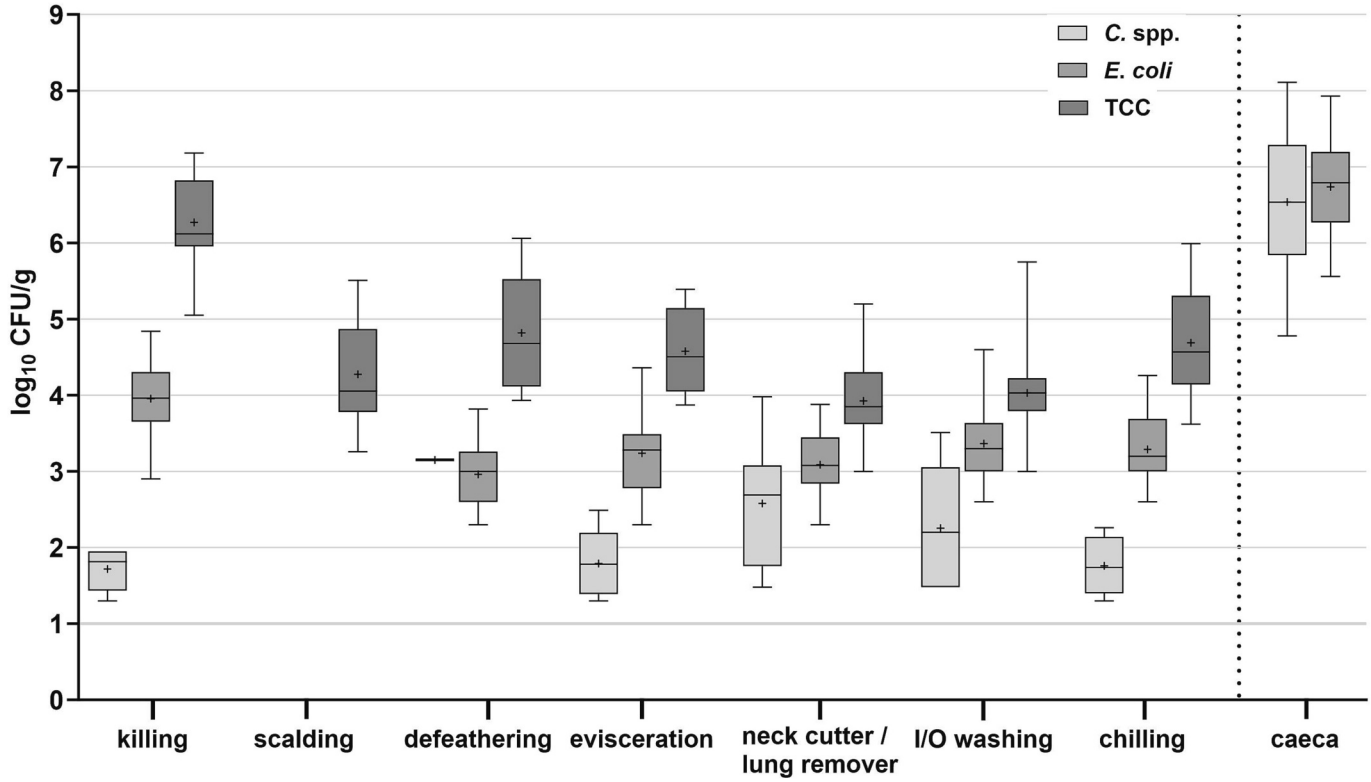

Fig. 5. Bacterial loads of *Campylobacter* spp., *E. coli* and TCC as log10 CFU/g after different stages of the turkey slaughtering process and within the caeca (no analysis of TCC). Shown are box plots as log10 CFU/g with the median (line), mean (+), interquartile range and 95 % confidence interval (n = 18). All samples with a value above 10 CFU/g were included in the analysis.

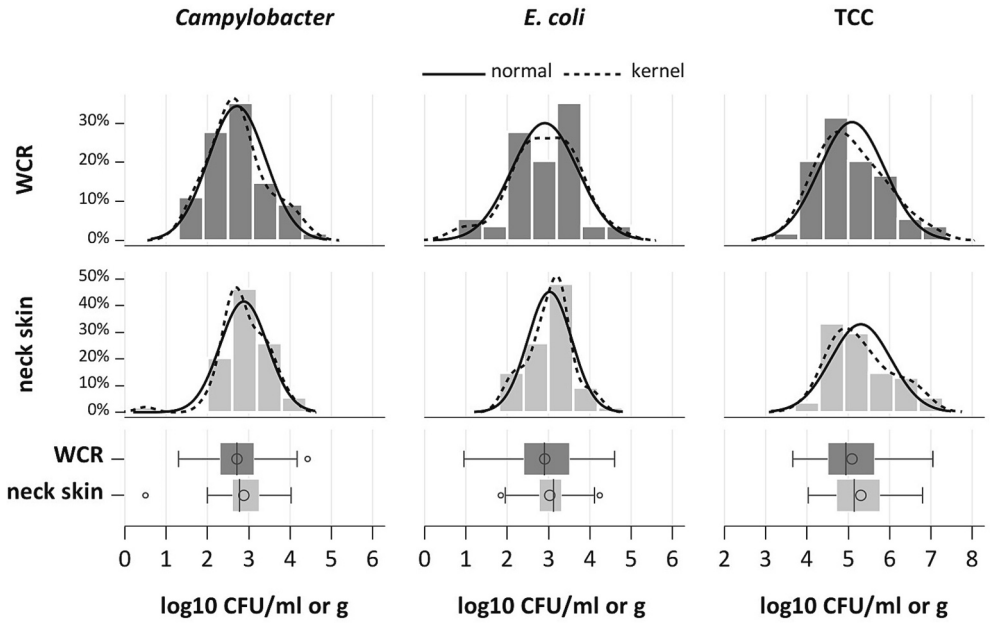

Fig. 6. Comparison of *Campylobacter* spp., *E. coli* and TCC counts of whole broiler carcass rinse samples (WCR) and neck skin samples taken after neck cutter/lung remover. Shown are the histograms with normal and kernel distribution and boxplots for both sampling methods and three bacterial counts.

indicator or to predict the level of *Campylobacter* contamination.

Studies on the correlation between *Campylobacter*, *E. coli*, and TCC levels during poultry slaughtering processes have produced varying results. Pacholewicz et al. (2015a) observed similar trends in changes along the processing line for *E. coli* and *Campylobacter*, suggesting *E. coli* as a potential hygiene indicator in broiler processing. Consistent with these findings, the present study showed a significant correlation between the changes in the three bacteria levels in broiler slaughterhouses, though the correlations found were not strong. Berrang and Dickens (2000) found a correlation coefficient of $R^2 = 0.7$ between TCC values and *Campylobacter*. Williams and Ebel (2014) identified a weak ($R^2 = 0.27$) but statistically significant correlation between TCC and

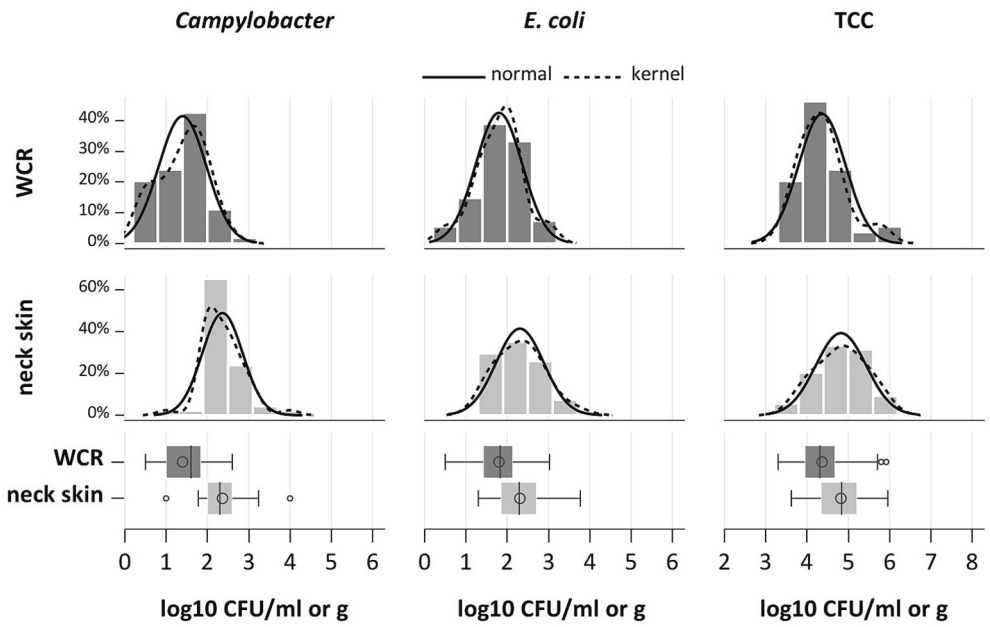

Fig. 7. Comparison of *Campylobacter* spp., *E. coli* and TCC counts of whole broiler carcass rinse samples (WCR) and neck skin samples taken after chilling. Shown are the histograms with normal and kernel distribution and boxplots for both sampling methods and three bacterial counts.

Table 2
Comparison of WCR and neck skin samples after two processing steps neck cutter/lung remover and after chilling in three different broiler slaughterhouses. Shown are the results of Student's *t*-Tests and F-Tests of equality of variances, bold font indicates a significant difference between WCR and neck skin.

Bacteria	Station	Sample type	Mean + SD (log_{10} cfu/ml or g)	Mean difference (log_{10} cfu/ml or g)	*p*-Value
Campylobacter	After lung remover	Neck skin	2.9 ± 0.4	0.2	0.1823
		WCR	2.7 ± 0.5		
	After chilling	Neck skin	2.4 ± 0.8	**1.0**	**<0.0001**
		WCR	1.4 ± 0.4		
E. coli	After lung remover	Neck skin	3.0 ± 0.3	0.1	0.3559
		WCR	2.9 ± 0.6		
	After chilling	Neck skin	2.3 ± 0.4	**0.5**	**<0.0001**
		WCR	1.8 ± 0.4		
TCC	After lung remover	Neck skin	5.3 ± 0.4	0.2	0.1441
		WCR	5.1 ± 0.4		
	After chilling	Neck skin	4.8 ± 0.3	**0.6**	**<0.0001**
		WCR	4.4 ± 0.4		

Campylobacter. Moreover, Duffy et al. (2014) reported a strong correlation ($R^2 = 0.8$) between *E. coli* and *Campylobacter* concentrations in broiler carcasses, considering multiple sampling points. Our findings are therefore in line with those of previous studies. However, in turkeys, no significant correlation was observed between the two bacterial species and TCC. A reason could be the lower sample size compared to the broiler dataset as well as the lower number of *Campylobacter* positive samples. Overall, based on the low correlation strength in this study, enumeration of *E. coli* as well as TCC offered limited benefit for the prediction of *Campylobacter* contamination levels of broiler carcasses.

4. Conclusion

Contamination of both broilers and turkeys decreased during the slaughtering process in this study. In order to achieve further reductions at the end of the slaughter chain and thus maximally reduce consumer exposure to *Campylobacter* through poultry meat, processing steps such as evisceration or plucking as critical points for meat contamination should be further optimized and new technologies for reducing the bacterial contamination need to be developed and investigated.

Funding

The project was supported by funds of the Federal Ministry of Food and Agriculture, Germany (BMEL) based on a decision of the Parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE) under the innovation support programme (grant number FKZ 281C104B18) and by the Federal Ministry of Education and Research, Germany (BMBF, grant number IP2/01KI2007A).

CRediT authorship contribution statement

A. Beterams: Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Conceptualization. **C. Püning:** Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Conceptualization. **B. Wyink:** Investigation, Methodology, Writing – review & editing. **J. Grosse-Kleimann:** Formal analysis, Investigation, Writing – review & editing. **G. Gölz:** Investigation, Supervision, Writing – review & editing. **A. Schönknecht:** Investigation, Supervision, Writing – review & editing. **T. Alter:** Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing. **F. Reich:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Supervision, Writing – review & editing.

Declaration of competing interest

None.

Data availability

The data that has been used is confidential.

References

Alter, T., Scherer, K., 2006. Stress response of *Campylobacter* spp. and its role in food processing. J. Vet. Med. B Infect. Dis. Vet. Public Health. 53, 351–357. https://doi.org/10.1111/j.1439-0450.2006.00983.x.

Allen, V.M., Bull, S.A., Corry, J.E., Domingue, G., Jørgensen, F., Frost, J.A., Whyte, R., Gonzalez, A., Elvis, N., Humphrey, T.J., 2007. *Campylobacter* spp. contamination of chicken carcasses during processing in relation to flock colonisation. Int. J. Food Microbiol. 113 (1), 54–61. https://doi.org/10.1016/j.ijfoodmicro.2006.07.011.

Alter, T., Gaull, F., Froeb, A., Fehlhaber, K., 2005. Distribution of *Campylobacter jejuni* strains at different stages of a turkey slaughter line. Food Microbiol. 22, 345–351. https://doi.org/10.1016/j.fm.2004.08.008.

Atanassova, V., Reich, F., Beckmann, L., Klein, G., 2007. Prevalence of *Campylobacter* spp. in turkey meat from a slaughterhouse and in turkey meat retail products. FEMS Microbiol. Immunol. 49, 141–145. https://doi.org/10.1111/j.1574-695X.2006.00180.x.

Berrang, M.E., Bailey, J.S., 2009. On-line brush and spray washers to lower numbers of *Campylobacter* and *Escherichia coli* and presence of *Salmonella* on broiler carcasses during processing. J. Appl. Poultry Res. 18, 74–78. https://doi.org/10.3382/japr.2008-00067.

Berrang, M.E., Dickens, J.A., 2000. Presence and level of *Campylobacter* spp. on broiler carcasses throughout the processing plant. J. Appl. Poul. Res. 9, 43–47. https://doi.org/10.1093/japr/9.1.43.

Berrang, M.E., Buhr, R.J., Cason, J.A., Dickens, J.A., 2001. Broiler carcass contamination with *Campylobacter* from feces during defeathering. J. Food Prot. 64, 2063–2066. https://doi.org/10.4315/0362-028X-64.12.2063.

Blevins, R.E., Feye, K.M., Dittoe, D.K., Bench, L., Bench, B.J., Ricke, S.C., 2020. Aerobic plate count, *Salmonella* and *Campylobacter* loads of whole bird carcass rinses from pre-chillers with different water management strategies in a commercial poultry processing plant. J. Environ. Sci. Health B 55, 155–165. https://doi.org/10.1080/03601234.2019.1670522.

Buess, S., Zurfluh, K., Stephan, R., Guldimann, C., 2019. Quantitative microbiological slaughter process analysis in a large-scale Swiss poultry abattoir. Food Control 105, 86–93. https://doi.org/10.1016/j.foodcont.2019.05.012.

Chantarapanont, W., Berrang, M., Frank, J.F., 2003. Direct microscopic observation and viability determination of Campylobacter jejuni on chicken skin. J. Food Prot. 66, 2222–2230. https://doi.org/10.4315/0362-028X-66.12.2222.

Corry, J.E.L., Jarvis, B., Passmore, S., Hedges, A., 2007. A critical review of measurement uncertainty in the enumeration of food micro-organisms. Food Microbiol. 24, 230–253. https://doi.org/10.1016/j.fm.2006.05.003.

Dogan, O.B., Aditya, A., Ortuzar, J., Clarke, J., Wang, B., 2022. A systematic review and meta-analysis of the efficacy of processing stages and interventions for controlling *Campylobacter* contamination during broiler chicken processing. Compr. Rev. Food Sci. F. 21, 227–271. https://doi.org/10.1111/1541-4337.12860.

Duffy, L.L., Blackall, P.J., Cobbold, R.N., Fegan, N., 2014. Quantitative effects of in-line operations on *Campylobacter* and *Escherichia coli* through two Australian broiler processing plants. Int. J. Food Microbiol. 188, 128–134. https://doi.org/10.1016/j.ijfoodmicro.2014.07.024.

EFSA, 2011. Scientific opinion on *Campylobacter* in broiler meat production: control options and performance objectives and/or targets at different stages of the food chain. EFSA J. 9 https://doi.org/10.2903/j.efsa.2011.2105.

EFSA, 2022. The European Union one health 2021 Zoonoses report. EFSA J. 20 https://doi.org/10.2903/j.efsa.2022.7666.

Elvers, K.T., Morris, V.K., Newell, D.G., Allen, V.M., 2011. Molecular tracking, through processing, of *Campylobacter* strains colonizing broiler flocks. Appl. Environ. Microbiol. 77, 5722–5729. https://doi.org/10.1128/aem.02419-10.

Gill, C.O., Badoni, M., 2005. Recovery of bacteria from poultry carcasses by rinsing, swabbing or excision of skin. Food Microbiol. 22, 101–107. https://doi.org/10.1016/j.fm.2004.04.005.

Guerin, M.T., Sir, C., Sargeant, J.M., Waddell, L., O'Connor, A.M., Wills, R.W., Bailey, R. H., Byrd, J.A., 2010. The change in prevalence of *Campylobacter* on chicken carcasses during processing: a systematic review. Poult. Sci. 89, 1070–1084. https://doi.org/10.3382/ps.2009-00213.

Hauge, S.J., Johannessen, G.S., Haverkamp, T.H.A., Bjorkoy, S., Llarena, A.K., Spilsberg, B., Leithaug, M., Okland, M., Holthe, J., Rotterud, O.J., Alvseike, O., Nagel-Alne, G.E., 2023. Assessment of poultry process hygiene and bacterial dynamics along two broiler slaughter lines in Norway. Food Control 146, 109526. https://doi.org/10.1016/j.foodcont.2022.109526.

Hutchison, M.L., Harrison, D., Tchorzewska, M.A., Gonzalez-Bodi, S., Madden, R.H., Corry, J.E.L., Allen, V.M., 2022. Quantitative determination of *Campylobacter* on broilers along 22 United Kingdom processing lines to identify potential process control points and cross-contamination from colonized to uncolonized flocks. J. Food Prot. 85, 1696–1707. https://doi.org/10.4315/JFP-22-204.

Kim, S.H., Chelliah, R., Ramakrishnan, S.R., Perumal, A.S., Bang, W.S., Rubab, M., Daliri, E.B.M., Barathikannan, K., Elahi, F., Park, E., Jo, H.Y., Hwang, S.B., Oh, D.H., 2021. Review on stress tolerance in *Campylobacter jejuni*. Front. Cell Inf. Microbiol. 10, 596570 https://doi.org/10.3389/fcimb.2020.596570.

Lindblad, M., Hansson, I., Vagsholm, I., Lindqvist, R., 2006. Postchill *Campylobacter* prevalence on broiler carcasses in relation to slaughter group colonization level and chilling system. J. Food Prot. 69, 495–499. https://doi.org/10.4315/0362-028x-69.3.495.

Marmion, M., Ferone, M.T., Whyte, P., Scannell, A.G.M., 2021. The changing microbiome of poultry meat; from farm to fridge. Food Microbiol. 99, 103823 https://doi.org/10.1016/j.fm.2021.103823.

Musgrove, M.T., Cason, J.A., Fletcher, D.L., Stern, N.J., Cox, N.A., Bailey, J.S., 1997. Effect of cloacal plugging on microbial recovery from partially processed broilers. Poult. Sci. 76, 530–533. https://doi.org/10.1093/ps/76.3.530.

Nagel Gravning, G.E., Rotterud, O.J., Bjorkoy, S., Forseth, M., Skjerve, E., Llarena, A.K., Lian, A., Johannessen, G.S., Hauge, S.J., 2021. Comparison of four sampling methods for microbiological quantification on broiler carcasses. Food Control 121, 107589. https://doi.org/10.1016/j.foodcont.2020.107589.

Nauta, M., Van der Fels-Klerx, I., Havelaar, A., 2005. A poultry-processing model for quantitative microbiological risk assessment. Risk Anal. 25, 85–98. https://doi.org/10.1111/j.0272-4332.2005.00569.x.

Northcutt, J.K., Smith, D.P., Musgrove, M.T., Ingram, K.D., Hinton, A., 2005. Microbiological impact of spray washing broiler carcasses using different chlorine concentrations and water temperatures. Poult. Sci. 84, 1648–1652. https://doi.org/10.1093/ps/84.10.1648.

Pacholewicz, E., Liakopoulos, A., Swart, A., Gortemaker, B., Dierikx, C., Havelaar, A., Schmitt, H., 2015a. Reduction of extended-spectrum-beta-lactamase- and AmpC-beta-lactamase-producing *Escherichia coli* through processing in two broiler chicken slaughterhouses. Int. J. Food Microbiol. 215, 57–63. https://doi.org/10.1016/j.ijfoodmicro.2015.08.010.

Pacholewicz, E., Swart, A., Schipper, M., Gortemaker, B.G.M., Wagenaar, J.A., Havelaar, A.H., Lipman, L.J.A., 2015b. A comparison of fluctuations of *Campylobacter* and *Escherichia coli* concentrations on broiler chicken carcasses during processing in two slaughterhouses. Int. J. Food Microbiol. 205, 119–127. https://doi.org/10.1016/j.ijfoodmicro.2015.04.006.

Pacholewicz, E.W.A., Swart, A., Wagenaar, J.A., Lipman, L.J.A., Havelaar, A.H., 2016. Explanatory variables associated with *Campylobacter* and *Escherichia coli* concentrations on broiler chicken carcasses during processing in two slaughterhouses. J. Food Prot. 79, 2038–2047. https://doi.org/10.4315/0362-028x.Jfp-16-064.

Park, S.F., 2002. The physiology of *Campylobacter* species and its relevance to their role as foodborne pathogens. Int. J. Food Microbiol. 74, 177–188. https://doi.org/10.1016/S0168-1605(01)00678-X.

Perez-Arnedo, I., Gonzalez-Fandos, E., 2019. Prevalence of *Campylobacter* spp. in poultry in three spanish farms, a slaughterhouse and a further processing plant. Foods 8, 111. https://doi.org/10.3390/foods8030111.

Rasschaert, G., De Zutter, L., Herman, L., Heyndrickx, M., 2020. *Campylobacter* contamination of broilers: the role of transport and slaughterhouse. Int. J. Food Microbiol. 322, 108564 https://doi.org/10.1016/j.ijfoodmicro.2020.108564.

Reich, F., Atanassova, V., Haunhorst, E., Klein, G., 2008. The effects of *Campylobacter* numbers in caeca on the contamination of broiler carcasses with *Campylobacter*. Int. J. Food Microbiol. 127, 116–120. https://doi.org/10.1016/j.ijfoodmicro.2008.06.018.

Rosenquist, H., Sommer, H.M., Nielsen, N.L., Christensen, B.B., 2006. The effect of slaughter operations on the contamination of chicken carcasses with thermotolerant *Campylobacter*. Int. J. Food Microbiol. 108, 226–232. https://doi.org/10.1016/j.ijfoodmicro.2005.12.007.

Sánchez, M.X., Fluckey, W.M., Brashears, M.M., McKee, S.R., 2002. Microbial profile and antibiotic susceptibility of *Campylobacter* spp. and *Salmonella* spp. in broilers processed in air-chilled and immersion-chilled environments. J. Food Prot. 65, 948–956. https://doi.org/10.4315/0362-028X-65.6.948.

Seliwiorstow, T., Bare, J., Van Damme, I., Uyttendaele, M., De Zutter, L., 2015. *Campylobacter* carcass contamination throughout the slaughter process of *Campylobacter*-positive broiler batches. Int. J. Food Microbiol. 194, 25–31. https://doi.org/10.1016/j.ijfoodmicro.2014.11.004.

Son, I., Englen, M.D., Berrang, M.E., Fedorka-Cray, P.J., Harrison, M.A., 2007. Prevalence of *Arcobacter* and *Campylobacter* on broiler carcasses during processing. Int. J. Food Microbiol. 113, 16–22. https://doi.org/10.1016/j.ijfoodmicro.2006.06.033.

Stern, N.J., 2008. *Salmonella* species and *Campylobacter jejuni* cecal colonization model in broilers. Poult. Sci. 87, 2399–2403. https://doi.org/10.3382/ps.2008-00140.

Szott, V., Reichelt, B., Friese, A., Roesler, U., 2022. A complex competitive exclusion culture reduces *Campylobacter jejuni* colonization in broiler chickens at slaughter age in vivo. Vet. Sci. 9. Article 181 https://doi.org/10.3390/vetsci9040181.

Tondeur, W., Simons, P., van Schie, T., Hollemann, J., 2019. Broiler Meat Signals: A Practical Guide to Improving Poultry Meat Quality. Roodbont Publisher B.V, Zutphen, NL.

Williams, M.S., Ebel, E.D., 2014. Estimating the correlation between concentrations of two species of bacteria with censored microbial testing data. Int. J. Food Microbiol. 175, 1–5. https://doi.org/10.1016/j.ijfoodmicro.2014.01.007.

Yusufu, H.I., Genigeorgis, C., Farver, T.B., Wempe, J.M., 1983. Prevalence of *Campylobacter jejuni* at different sampling sites in two California turkey processing plants. J. Food Prot. 46, 868–872. https://doi.org/10.4315/0362-028X-46.10.868.

Zhang, Q.Q., Ye, K.P., Xu, X.L., Zhou, G.H., Cao, J.X., 2012. Comparison of excision, swabbing and rinsing sampling methods to determine the microbiological quality of broiler carcasses. J. Food Safety 32, 134–139. https://doi.org/10.1111/j.1745-4565.2011.00360.x.

Zhang, X.B., Peng, Z.X., Li, P., Mao, Y.W., Shen, R., Tao, R., Diao, X.G., Liu, L.H., Zhao, Y. Z., Luo, X., 2020. Complex internal microstructure of feather follicles on chicken skin promotes the bacterial cross-contamination of carcasses during the slaughtering process. Front. Microbiol. 11. Article 571913 https://doi.org/10.3389/fmicb.2020.571913.