Analysis of a poultry slaughter process: Influence of process stages on the microbiological contamination of broiler carcasses

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Abstract

In a large-scale Swiss poultry abattoir, a microbiological process analysis of broiler carcasses was performed. At each selected process stage (scalding, plucking, evisceration, washing, and chilling), 90 carcasses from 30 flocks were sampled and examined for *Campylobacter*, *Salmonella*, *Escherichia coli*, Enterobacteriaceae, and extended-spectrum β-lactamases-producing Enterobacteriaceae. With regard to *Campylobacter* counts on carcasses, plucking tended to slightly increase the results (on average by 0.4 log CFU/g), whereas mean counts from plucked and chilled carcasses were comparable (3.1 log CFU/g after plucking, 3.0 log CFU/g in the chiller). The *Campylobacter* results of chilled carcasses are thereby likely to comply with the newly defined requirements of the European Union (process hygiene criterion for *Campylobacter*). With regard to *Escherichia coli* and Enterobacteriaceae counts on carcasses, plucking clearly reduced the results (on average by 0.8 and 0.9 log CFU/g), whereas mean counts from plucked and chilled carcasses were comparable (3.4 and 3.5 log CFU/g after plucking, 3.4 log CFU/g in the chiller). In contrast, *Salmonella* spp. were not detected on broiler carcasses and extended-spectrum β-lactamases-producing Enterobacteriaceae only rarely (1.8%). Such abattoir-specific data are of central importance for assessment of slaughter process performance and if necessary for the implementation of effective measures in the slaughter process.

Introduction

The slaughter of poultry in large-scale slaughterhouses is a rapid and highly automated process. Despite technological advancements, there are still considerable opportunities for contamination and spread of bacteria during slaughter. To ensure food safety, strict adherence to good practices of slaughter hygiene, along with risk-based preventive measures (HACCP approach), is of central importance. For assessment of slaughter process performance, a process analysis including the identification of operations increasing or decreasing the microbiological contamination of carcasses is required (Brown *et al.,* 2000; Milios *et al.,* 2014; Zweifel *et al.,* 2014).

With regard to slaughtered broilers, such examinations will soon include also *Campylobacter*, which is the leading cause of acute bacterial gastroenteritis in humans (EFSA/ECDC, 2016). Handling and consumption of poultry are thereby considered major sources for human illnesses (Boysen *et al.,* 2014; EFSA, 2010a). Various risk assessments indicate that reductions of *Campylobacter* counts on carcasses might cause a significant reduction in associated human cases (EFSA, 2011; Nauta *et al.,* 2009; Rosenquist *et al.,* 2003). Moreover, certain poultry slaughterhouses are more successful than others in containing the *Campylobacter* contamination of carcasses (EFSA, 2010b; Habib et *al.,* 2012a).

With the required monitoring of *Salmonella* and the planned introduction of *Campylobacter* as process hygiene criteria for slaughtered broilers in the European Union (EU), microbiological data are also required for the implementation of adequate measures in the slaughter process. The present study was performed in a large-scale poultry abattoir and the aim was to investigate the effects of selected slaughter operations on the microbiological contamination of broiler carcasses (selected foodborne pathogens and indicator bacteria).

Materials and Methods

Abattoir and slaughter process

This study was based on investigations carried out during May to June 2017 in a large-scale Swiss poultry abattoir. Most processing steps were automated. First, broilers were stunned using CO_2, manually shackled, and exsanguinated. Broilers were then scalded (immersion) and plucked. Scalding comprised two scalding tanks (tank 1: on average 52.4°C, 120 s; tank 2: on average 52.5°C, 75 s) and plucking consisted of two segments. After transfer to the evisceration line, intestines were drawn out of the body's cavity (vent cutter, opener, eviscerator), the neck was removed, and washing steps with cold potable water removed visible dirt. Finally, carcasses were chilled.

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Sampling

Broiler carcasses were sampled at five slaughter process stages: after scalding, after plucking, after evisceration, after washing, and in the chiller. At each stage, 90 carcasses from 30 flocks were sampled during 10 sampling days. Each carcass sample consisted of pooled neck and breast skin. In addition, 30 scalding water samples (15 per scalding tank) were collected during five sampling days.

Microbiological examinations

Samples were analyzed qualitatively for *Salmonella* spp. and quantitatively for *Campylobacter* spp., *Escherichia* (*E.*) *coli*, Enterobacteriaceae, and extended-spectrum β-lactamases (ESBL)-producing Enterobacteriaceae. The qualitative examination for *Salmonella* spp. was done in accordance with ISO 6579:2007-10 with a modification. Briefly, a subset of each carcass sample (10 g) or of each scalding water sample (10 mL) was enriched (24 h, 37°C) at a 1:10 ratio in buffered peptone water (Oxoid, Pratteln, CH). From the first enrichment, 0.1 mL were incubated (24 h, 41.5°C) in 10 mL of Rappaport-Vassiliadis broth (Oxoid). The enriched samples were subcultured (24 h, 37°C) on xylose-lysine-desoxycholate (XLD) agar (Bio-Rad, Reinach,

CH) and mannitol lysine crystal violet brilliant green (MLCB) agar (Oxoid). For the quantitative examinations, a subset of each carcass sample (10 g) was homogenized at a 1:10 ratio in 0.85% saline solution and analyzed using a spiral plater (Eddy Jet, IUL, Barcelona, E). Scalding water samples were quantitatively analyzed by spreading 0.1 mL. The following agars and conditions were used for the quantitative examinations: CampyFood agar (bioMérieux, Geneva, CH; 48 h, 41.5°C, microaerophilic conditions), Rapid *E. coli* 2™ agar (Bio-Rad; 24 h, 37°C), violet red bile glucose (VRBG) agar (Becton Dickinson, Allschwil, CH; 48 h, 30°C, anaerobic conditions), and chromogenic Brilliance ESBL agar (Oxoid; 24 h, 37°C).

Data analysis

Colony counts of quantitatively analyzed samples were expressed as log CFU/g or log CFU/mL. The detection limit was 200 CFU/g (2.3 log CFU/g) for carcass samples and 10 CFU/mL (1.0 log CFU/mL) for scalding water samples. Quantitative analysis was based on counts above the detection limit and results were compared by reference to mean log values. Mean log values differing by <0.5 log units were regarded as similar for practical purposes. Statistical analysis was performed using JMP 13.0 (SAS Institute, Cary, NC, USA). The level of significance was set at α=0.05. Analysis of variance (ANOVA) and Tukey HSD test were used to analyze differences in counts between sequential process stages and scalding segments.

Results and Discussion

Microbiological slaughter process analysis: *Salmonella* spp. and *Campylobacter* spp.

While *Salmonella* spp. were not detected (after enrichment) in any of the 450 samples from broiler carcasses, 131 (29%) of all carcasses showed *Campylobacter* counts above the detection limit. At the different process stages, the proportion *Campylobacter*-positive carcasses ranged from 11 to 42% (Table 1). Because consumer risks seem mainly associated with highly contaminated products, the distribution of *Campylobacter* counts at different ranges is of importance. The distribution of counts at different ranges is shown in Table 1. Over all process stages, 50% of the *Campylobacter*-positive carcasses showed counts <3.0 log CFU/g and 42% between 3.0 and 4.0 log CFU/g. After scalding, *Campylobacter* counts averaged out at 2.7 log CFU/g (Table 1, Figure 1). Plucking tended to increase and washing to reduce the counts (on average by 0.4 log CFU/g), whereas results remained mainly constant by evisceration and chilling. The resulting mean count of chilled carcasses was 3.0 log CFU/g. With regard to *Campylobacter* and poultry slaughter, the available literature often showed common trends: reductions by scalding, rather increases by plucking, no changes or increases by evisceration, and reductions by washing and chilling (Berghaus *et al.,* 2013; Duffy *et al.,* 2014; Guerin *et al.,* 2010; Huang *et al.,* 2017; Pacholewicz *et al.,* 2015b; Rosenquist *et al.,* 2006; Seliwiorstow *et al.,* 2015). However, direct comparisons are often hampered because the effect of a certain process stage on *Campylobacter* strongly depends on the respective conditions (*e.g.* temperature and time conditions used for scalding, varying washing steps, differing chilling methods).

Furthermore, microbiological criteria for *Campylobacter* spp. on slaughtered broilers have been recently investigated (Comin *et al.,* 2014; EFSA, 2011; Lee *et al.,* 2015; Nauta *et al.,* 2012). With a new amendment of Reg. (EC) No. 2073/2005 (EC, 2005), the EU plans to implement a quantitative process hygiene criterion for *Campylobacter* on broiler carcasses after chilling. The amendment lays down requirements in view of sampling, sampling plans, sample processing, microbiological examinations, evaluation of results, and corrective actions (Table 2). *Campylobacter* results are thereby rated as satisfactory if not more than 20 (40%) of 50 samples (one sample consists of three neck skin samples from one flock) from 10 consecutive samplings show counts >1000 CFU/g (n=50, c=20). Thereby, it must be considered that i) countries with more favorable *Campylobacter* contamination levels may apply stricter c values and ii) c values will be reduced 2020 (c=15) and 2025 (c=10). Although the design of the present study does not fully correspond with the EU requirements, the results of the chilled carcasses were evaluated on the basis of the defined EU limits. Of the 90 chilled broiler carcasses (Table 1), only 12 (13%) showed

Table 1. *Campylobacter, Escherichia coli*, and Enterobacteriaceae results from broiler carcasses at selected stages of slaughter (n=90 at each process stage, sampling comprised 10 sampling days and a total of 30 broiler flocks).

Microorganisms	Process stage	Results ≥ detection limit		Counts[a]		Number (%) of carcasses with counts ≥detection limit at different ranges (log CFU/g)[b]			
		Carcasses, %	Flocks, %	χ	SD	<3.0	3.0-4.0	4.0-5.0	>5.0
Campylobacter	After scalding	11.1	26.7	2.69	0.39	8 (80.0)	2 (20.0)	0 (0.0)	0 (0.0)
	After plucking	42.2	53.3	3.13	0.63	17 (44.7)	18 (47.4)	3 (7.9)	0 (0.0)
	After evisceration	41.1	50.0	3.20	0.68	14 (37.8)	17 (45.9)	6 (16.2)	0 (0.0)
	After washing	23.3	40.0	2.77	0.45	13 (61.9)	8 (38.1)	0 (0.0)	0 (0.0)
	In the chiller	27.8	36.7	2.99	0.60	13 (52.0)	10 (40.0)	2 (8.0)	0 (0.0)
E. coli	After scalding	94.4	100	4.16	1.08	12 (14.1)	21 (24.7)	35 (41.2)	17 (20.0)
	After plucking	92.2	100	3.37	0.88	31 (37.3)	33 (39.8)	12 (14.5)	7 (8.4)
	After evisceration	96.7	100	3.75	0.73	11 (12.6)	42 (48.3)	28 (32.2)	6 (6.9)
	After washing	91.1	100	3.34	0.74	29 (35.4)	37 (45.1)	14 (17.1)	2 (2.4)
	In the chiller	82.2	96.7	3.41	0.75	22 (29.7)	38 (51.4)	12 (16.2)	2 (2.7)
Enterobacteriaceae	After scalding	90.0	96.7	4.42	1.01	6 (7.4)	19 (23.5)	36 (44.4)	20 (24.7)
	After plucking	93.3	96.7	3.52	0.86	22 (26.2)	38 (45.2)	18 (21.4)	6 (7.1)
	After evisceration	97.8	100	3.83	0.69	8 (9.1)	43 (48.9)	31 (35.2)	6 (6.8)
	After washing	94.4	100	3.34	0.76	29 (34.1)	38 (44.7)	15 (17.6)	3 (3.5)
	In the chiller	87.8	100	3.39	0.74	22 (27.8)	41 (51.9)	14 (17.7)	2 (2.5)

[a]χ and SD, mean log CFU/g and standard deviation of results ≥detection limit (2.3 log CFU/g). [b]*Campylobacter*: 131 carcasses with ≥2.3 log CFU/g, *E. coli*: 411 carcasses with ≥2.3 log CFU/g, Enterobacteriaceae: 417 carcasses with ≥2.3 log CFU/g.

Campylobacter counts ≥1000 CFU/g and only nine (10%) of them exceeded 1000 CFU/g. Looking at sets of consecutive 50 samples (*moving window*: samples 1-50, 6-55, etc.), in each case only five (10%) exceeded 1000 CFU/g. Thus, the *Campylobacter* results of chilled carcasses likely comply with the EU limits. First experiences with a newly implemented *Campylobacter* performance target using to a certain degree a similar approach have recently been reported from New Zealand (Lee *et al.,* 2015).

Microbiological slaughter process analysis: *Escherichia coli* and Enterobacteriaceae

E. coli and Enterobacteriaceae were used as indicator of fecal contamination on broiler carcasses. Of the 450 carcasses, 91% and 93% showed counts above the detection limit for *E. coli* and Enterobacteriaceae, respectively. The distribution of counts at different ranges is shown in Table 1. During the slaughter process, trends and counts were comparable for *E. coli* and Enterobacteriaceae (Figure 1). Enterobacteriaceae were therefore mainly *E. coli.*

After scalding, *E. coli* and Enterobacteriaceae counts averaged out at 4.2 and 4.4 log CFU/g, respectively (Table 1, Figure 1). Plucking reduced the counts (on average by 0.8 and 0.9 log CFU/g; P<0.05), probably due to physical removal. Pacholewicz *et al.* (2015b) recently also reported reductions by plucking, whereas an earlier study described an opposite effect (Berrang and Dickens, 2000). In the present study, evisceration slightly increased the counts (on average by 0.4 and 0.3 log CFU/g; P<0.05 for *E. coli*), whereas washing tended to reduce the counts (on average by 0.4 and 0.5 log CFU/g; P<0.05). Thus, as described previously (Berrang and Dickens, 2000; Pacholewicz *et al.,* 2015b), evisceration operations were performed without extensive additional fecal contamination. By washing with cold water probably rather redistributions than real reductions were achieved (Loretz *et al.,* 2010). Using air chilling with an usually limited microbial effect (James *et al.,* 2006), results remained mainly constant and resulting mean *E. coli* and Enterobacteriaceae counts of chilled carcasses were 3.4 log CFU/g.

Microbiological slaughter process analysis: extended-spectrum β-lactamases-producing *Enterobacteriaceae*

With regard to antibiotic resistance, ESBL-producing Enterobacteriaceae are currently of special concern (Seiffert *et al.,* 2013). With regard to foods of animal origin, in particular healthy chickens as carriers and contaminated poultry products are

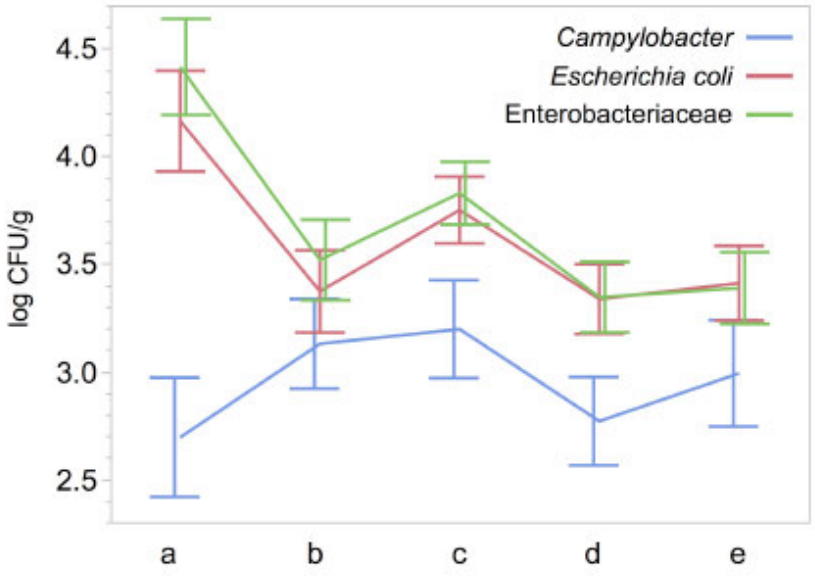

Figure 1. Mean *Campylobacter, Escherichia coli*, and Enterobacteriaceae counts from broiler carcasses with results ≥2.3 log CFU/g: (a) after scalding, (b) after plucking, (c) after evisceration, (d) after washing, and (e) in the chiller (error bars represent 95% confidence intervals).

Table 2. Planned process hygiene criterion for *Campylobacter* on broiler carcasses after chilling in the European Union (amendment to Reg. [EC] No. 2073/2005).

	Microorganisms	Sampling plan[a] n	c	Limits	Action in case of unsatisfactory results
Carcasses of broilers (after chilling)	*Campylobacter*[b]	50	20[c]	1000 CFU/g	Improvements in slaughter hygiene, review of process controls, origin of animals and of the biosecurity measures in the farms of origin

[a]n = No. of samples from 10 consecutive sampling sessions, c = No. of samples with *Campylobacter* counts >1000 CFU/g. [b]Satisfactory, if a maximum of c/n samples show values >1000 CFU/g; unsatisfactory, if more than c/n samples show values >1000 CFU/g. [c]From 1.1.2020: c = 15, from 1.1.2025: c = 10.

Table 3. *Campylobacter, Escherichia coli*, and Enterobacteriaceae results from scalding water samples (n=30).

Scalding tanka	*Campylobacter*[b]			*E. coli*			Enterobacteriaceae		
	n pos	χ	SD	n pos	χ	SD	n pos	χ	SD
No. 1 (n = 15)	7	2.46	0.94	15	4.11	0.69	15	4.02	0.47
No. 2 (n = 15)	10	1.47	0.57	15	3.07	0.58	15	3.18	0.52

[a]Tank 1: on average 52.4°C, 120 s, tank 2: on average 52.5°C, 75 s. [b]n pos, No. of samples with results ≥ detection limit (1.0 log CFU/mL); χ and SD, mean log CFU/mL and standard deviation of results ≥ detection limit.

currently in the focus (Abgottspon *et al.,* 2014; Geser *et al.,* 2012). A high prevalence of 63% (flock level) was recently reported by examining chicken fecal samples collected at slaughter with an enrichment step (Geser *et al.,* 2012). In the present study, ESBL-producing Enterobacteriaceae (CTX-1 producing *Escherichia coli* and CTX-1 producing *Escherichia fergusonii*) were found quantitatively on only eight (1.8%) broiler carcasses (seven from one sampling day and six from the same flock) and counts ranged from 2.3 to 3.9 log CFU/g. None of the final chilled carcasses tested positive. In contrast, Pacholewicz *et al.* (2015a) could recently enumerate ESBL-producing *E. coli* in 82% of 620 samples collected trough processing in two broiler slaughterhouses in Germany and the Netherlands.

Scalding water

In the examined poultry abattoir, a two-segment scalding system was used. Such systems are intended to expose the carcasses to less and less contaminated scalding water (Cason *et al.,* 2000; Hinton *et al.,* 2004). In accordance, *Campylobacter*, *E. coli*, and *Enterobacteriaceae* counts were lower ($P<0.05$) in the second scalding tank than in the first scalding tank. Differences of mean *Campylobacter*, *E. coli*, and *Enterobacteriaceae* counts thereby accounted for 1.0, 1.0, and 0.8 log CFU/mL, respectively (Table 3). To assess the effect of scalding and the respective parameters (in particular exposition temperature and time), further investigations including carcasses before and after scalding are required. Furthermore, one scalding water sample was positive for *Salmonella* spp. and one for ESBL-producing Enterobacteriaceae. The *Salmonella* isolate was identified as *Salmonella enterica* subsp. *enterica* 4,12:i:–

Conclusions

A quantitative microbiological process analysis of broiler carcasses was performed at selected stages (scalding, plucking, evisceration, washing, chilling) in a large-scale poultry abattoir. *Campylobacter* spp. were found at the different stages on 11% (after scalding) to 42% (after plucking) of the carcasses. With regard to *Campylobacter* counts on carcasses, plucking tended to slightly increase the results (on average by 0.4 log CFU/g). Commonly minor changes occurred at the following stages and mean counts from plucked and chilled carcasses were similar (about 3.0 log CFU/g). Although not directly comparable, *Campylobacter* results of chilled carcasses are likely to comply with the newly defined EU requirements (process hygiene criterion for *Campylobacter*). *E. coli* and Enterobacteriaceae were found in remarkable frequencies and counts in the poultry slaughter process. With regard to *E. coli* and Enterobacteriaceae counts on carcasses, plucking clearly reduced the results (on average almost by one order of magnitude). Some process stage-specific changes were evident in the following, but mean *E. coli* and Enterobacteriaceae counts from plucked and chilled carcasses were similar (about 3.4 log CFU/g). On the other hand, *Salmonella* spp. and ESBL-producing Enterobacteriaceae were not or only rarely detected on the broiler carcasses. Such abattoir-specific microbiological data from carcasses form the basis for assessment of slaughter process performance, are of central importance for the implementation of HACCP-based systems, and allow if necessary (*e.g.* non-compliance with process hygiene criteria) to take targeted measures at selected slaughter process stages.

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