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Comparative examination of neck and breast skin samples of broilers after chilling

Vergleichende Untersuchungen von Halshaut- und Brusthautproben von Broilern nach der Kühlung

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Summary

For process control, Reg. (EC) No 2073/2005 lays down process hygiene criteria for *Salmonella* spp. and *Campylobacter* spp. on carcasses of broilers. The purpose of this study was to compare the microbiological condition of neck skin and breast skin samples of broilers after chilling. In total, 115 carcasses were sampled and from each carcass neck skin and breast skin samples were examined for aerobic plate count, Enterobacteriaceae count, *Salmonella* spp. (qualitative), and *Campylobacter* spp. (semi-quantitative and qualitatively by PCR). *Salmonella* could not be detected. Wilcoxon-test for paired samples showed significant differences between the sampling locations of each carcass for aerobic plate count (APC), Enterobacteriaceae count and *Campylobacter* spp., respectively. As assumed, APC values for neck skin samples of all microbial parameters were higher than the ones for breast skin samples. Campylobacter-positive PCR results were obtained from 77 carcasses. 72 carcasses out of these were positive in both sampling sites, which indicated a 93.5 % agreement of positive samples, and a kappa of 0.64. Therefore, breast skin could be used for detection of *Campylobacter* spp., although detection rates were significantly lower for breast skin ($p < 0.001$). The opportunity to use breast skin if not enough neck skin is available is given in Reg. (EC) No 2073/2005, but if breast skin is used widely, it must be considered that the detection rates would be lower due to the sampling location. To prevent future uncertainty and possible underestimation of Campylobacter rates in poultry the sampling site used should be an indispensable part to assess the microbial result.

Keywords: poultry, carcass, Campylobacter, process hygiene, slaughter

Zusammenfassung

Zur Kontrolle einer Kontamination von Geflügelfleisch mit Salmonellen und Campylobacter sieht die VO (EG) Nr. 2073/2005 eine Prozesshygienekontrolle von Geflügelkarkassen vor. In der hier vorgestellten Studie wurde Halshaut vergleichend zur Brusthaut von Geflügelkarkassen nach der Kühlung untersucht. Von den insgesamt 115 nach der Kühlung beprobten Tierkörpern wurden jeweils Halshaut- und Brusthautproben auf die Gesamtkeimzahl (GKZ), den Gehalt an Enterobacteriaceae (EB), qualitativ auf Salmonellen und semiquantitativ und qualitativ mit PCR auf Campylobacter-Keime untersucht. Salmonellen wurden nicht nachgewiesen. Der Wilcoxon-Test für verbundene Stichproben zeigte für die logarithmierten Werte der GKZ, der EB- und der Campylobacter-Nachweise jeweils signifikante Unterschiede zwischen den Nachweisraten auf der Halshaut und der Brusthaut desselben Tieres. Die Werte der Halshautproben lagen wie erwartet oberhalb derjenigen der Brusthautproben. Bei 77 Tierkörpern war die PCR Campylobacter-positiv. Anhand der PCR-Ergebnisse konnte eine Übereinstimmung zwischen beiden Probenarten bei 72 Tierkörpern nachgewiesen werden, was einer Übereinstimmung von 93,5 % und einem Kappa von 0,64 zwischen beiden Lokalisationen für *Campylobacter* spp. entspricht. Somit könnte auch Brusthautprobenmaterial für den Nachweis von *Campylobacter* spp. verwendet werden. Allerdings war die Nachweisrate bei den Brusthautproben signifikant niedriger als bei den Halshautproben ($p < 0,001$). Die Verwendung von Brusthaut als Probematerial sieht auch die VO (EG) Nr. 2073/2005 vor, wenn nicht ausreichend Halshaut vorhanden ist. Um einer Unsicherheit oder auch Unterschätzung bei der Bewertung des Campylobacter-Ergebnisses zuvorzukommen, sollte zukünftig die Probenlokalisation zusammen mit dem mikrobiologischen Ergebnis angegeben werden.

Schlüsselwörter: Geflügel, Schlachttierkörper, Campylobacter, Prozesshygiene, Schlachtung

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Introduction

Campylobacteriosis is the most important foodborne disease in the EU. The incidence increased since 2006, which is in contrast to Salmonellosis, which decreased from 2006 to 2013. In recent years, a slight increase of *Salmonella* cases was observed again (EFSA 2007, 2009, 2010a, 2011a, EFSA & ECDC 2017).

The consumption of poultry products can be an important source of infection for both foodborne diseases (Jacobs-Reitsma 1997, EFSA 2006, 2010b, Skarp et al. 2016). For *Salmonella* monitoring and vaccination programs exist in different production stages in the poultry chain. After conducting a baseline study regarding the contamination of broiler carcasses with *Campylobacter* spp. in the EU in 2008 (EFSA 2010 c) and interpretation of the results, a process hygiene criterion came into force at the slaughter stage as from 2018 (Reg. (EU) No 2017/1495 resp. Reg. (EC) No 2073/2005). For the process hygiene criterion, a minimum of 15 carcasses will be sampled per slaughterhouse and week. A total of 26 g neck skin as pool sample has to be taken for analysis from at least three poultry carcasses of one flock after chilling. For the baseline survey on Campylobacter on poultry carcasses results from neck skin and breast skin samples are reported (EFSA 2010b, c). Therefore, it is recommended to collect samples from other parts of the carcass if the amount of 26 g neck skin cannot be reached (Reg. (EU) No. 2017/1495). The distribution of *Campylobacter* spp. varies at the surface of the poultry carcass and it can be assumed, that neck and breast skin samples differ significantly (EFSA 2011b, Reg. (EU) No 2017/1495 recital 6).

To obtain more information regarding detection rates of *Campylobacter* spp. the following study was conducted to compare the detection rate at different sampling locations and to estimate if results from breast skin agree with those from neck skin.

Materials and Methods

In 2017, carcasses from 115 broilers after chilling were collected in a broiler processing plant. Each carcass was removed from the processing chain directly after the chilling, but before entering the dissection line. From each carcass neck skin and breast skin samples were taken with sterile instruments. Each sample was divided in two parts (A and B) for microbiological examination. Sample part A was diluted in Buffered Peptone Water (1:10) and then 0.05 ml of each dilution was dropped on agar plates. The aerobic plate count (APC) and the Enterobacteriaceae count (EC) were performed following the Official Collection of Methods of Analysis based on § 64 of the German Food and Feed Act (Lebensmittel- und Futtermittelgesetzbuch, LFGB): ASU L 06.00-19 2017-10 for APC and ASU L 06.00.25 1987-11 for EC. In addition, sample part A was examined for *Salmonella* spp. following ISO 6579-1:2017-07 with small modifications. Sample part B was used for the investigation of thermotolerant *Campylobacter* spp. in a semi-quantitative approach following ISO/TS 10272-3:2010 + Cor 1:2011. Firstly, dilution steps in selective media were prepared and one loop of each dilution was spread on selective agar plates. After an incubation in a microaerophilic condition all plates with visible bacterial growth were analysed, using the multiplex-PCR (DNA extraction from colony material) after Wang et al. (2009), confirming *Campylobacter* spp. All positive PCR-results were linked to the respective dilution steps and semi-quantitative results were calculated as most probable numbers per g (MPN/g).

Bacteriological results detected as colony forming units (cfu) were transformed to logarithmic values to the power of 10 to achieve normal distribution. Statistical analyses were carried out using IBM SPSS (version 24). Agreement between breast and neck skin samples was evaluated using kappa-statistics. With the Wilcoxon-test for paired samples differences between breast and neck skin samples were investigated.

Results

All examined carcasses were negative for *Salmonella* spp.

Mean APC was log 4.34 cfu/g in neck skin samples and log 4.15 cfu/g in breast skin samples, with highest amount in neck skin (log 5.99 cfu/g). Mean EC in neck skin was log 2.98 cfu/g and log 2.71 cfu/g in breast skin with 5.00 log cfu/g as highest in neck skin. Semi-quantitative results of *Campylobacter* spp. showed differences between the locations by a factor of around 10: 3720.46 MPN/g in neck skin compared to 382.14 MPN/g in breast skin. On the logarithmic scale, this difference was around log 0.5 (neck skin: log 1.7 cfu/g; breast skin: log 1.3 cfu/g).

The Wilcoxon-Test for paired samples showed significant differences between the two sampling locations for APC, EC and *Campylobacter* spp. (APC: $p = 0.001$; EC: $p < 0.001$; *Campylobacter* spp.: $p = 0.001$). The detection rate in breast skin samples was significantly lower than in neck skin samples (Tab. 1).

To investigate whether the results of breast skin and of neck skin, were in agreement regarding the detection of *Campylobacter* spp., PCR-results were compared. 77 out of the 115 sampled carcasses were positive for Campylobacter spp. For 72 carcasses out of these Campylobacter-positive carcasses PCR-results were *Campylobacter* spp. positive in both sampling locations, which indicated an amount of 93.5 %. This resulted in a kappa-index of $k = 0.64$, which describes a substantial agreement for PCR detection in both locations.

Discussion

In our study, we could show that the occurrence of *Campylobacter* spp. agreed for breast and neck skin samples in 115 broiler carcasses. However, we also found that the log cfu values were significantly higher in neck skin samples for APC, EC, and *Campylobacter* spp.. Baré et al. (2013) found the highest concentration of *Campylobacter* on neck skin samples (3.5 ± 1.1 $\log_{10}$ cfu/g) and the lowest on breast skin samples (3.0 ± 1.0 $\log_{10}$ cfu/g). This is in accordance with the results of our study where we found a variation up

TABLE 1: *Microbiological results in $\log_{10}$ cfu/g for neck and breast skin samples.*

examination	sample location	mean ($\bar{x}$)	minimum	maximum	p-value
APC	neck skin	4.34	3.20	5.99	p = 0.001
	breast skin	4.15	3.04	5.80	
EC	neck skin	2.98	2.30	5.00	p ≤ 0.001
	breast skin	2.71	2.30	3.85	
Campylobacter spp. semi-quantitative	neck skin	1.70*	–	–	p = 0.001
	breast skin	1.30*	–	–	

*Semi-quantitative counts were counted in MPN/g, only mean values were transformed in logarithmic scale.

to 0.5 $\log_{10}$ between the two sampling locations with lower results for breast skin samples, too.

For the baseline survey of Campylobacter on poultry carcasses in the EU pooled neck skin and breast skin samples were analysed (EFSA 2010b) and for the process hygiene criterion *Campylobacter* spp. according to Reg. (EC) No 2073/2005 neck skin or other parts of the carcass have to be taken for analysis. However, EFSA reports that differences between neck and breast skin may occur with a variation up to 1 $\log_{10}$ between the results using different carcass location for analysis (EFSA 2011b).

Pepe et al. (2009) showed positive Campylobacter PCR results for 20 out of 50 neck skin samples and for 14 out of 50 breast skin samples. In our study, we could show a higher accordance between these two sampling locations. Therefore, we can assume that the possibility to find Campylobacter positive samples by using breast skin instead of neck skin is given but because of much lower detection rates it will not be the appropriate site for sampling. The true amount of Campylobacter on breast skin and other skin parts of the carcass compared to neck skin is not predictable. Therefore, we think it is an important information to know if breast skin or other skin parts are used in pooled samples for the process hygiene criterion to prevent underestimation of Campylobacter rates.

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Conflict of interest

The corresponding author confirms that there are no conflicts of interest regarding the preparation of the manuscript.

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Laboratory standard procedures

ASU L 06.00-19 2017-10 adopting DIN 10161:2016-12: Microbiological analysis of meat and meat products – Aerobic count at 30 °C – Drop plating method; German version

ASU L 06.00.25 1987-11 adopting DIN 10164-2:1986-08: Microbiological examination of meat and meat products; determination of Enterobacteriaceae by the drop method; German version

ISO 6579-1:2017-07: Microbiology of the food chain – Horizontal method for the detection, enumeration and serotyping of Salmonella – Part 1: Detection of *Salmonella* spp.; German version EN ISO 6579-1:2017-07; German version

ISO/TS 10272-3:2010 + Cor 1:2011: Microbiology of food and animal fedding stuffs – Horizontal method for detection and enumeration of *Campylobacter* spp. – Part 3: Semi-quantitative method (consolidated version)

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