The ability to detect campylobacter presence and concentration using different chicken carcass samples

Ellis-Iversen, Johanne; Gantzhorn, Mette R.; Borck Høg, Birgitte; Foddai, Alessandro; Nauta, Maarten

Published in:
Food Control

Link to article, DOI:
[10.1016/j.foodcont.2020.107294](https://doi.org/10.1016/j.foodcont.2020.107294)

Publication date:
2020

Document Version
Peer reviewed version

[Link back to DTU Orbit](#)

Citation (APA):
Ellis-Iversen, J., Gantzhorn, M. R., Borck Høg, B., Foddai, A., & Nauta, M. (2020). The ability to detect campylobacter presence and concentration using different chicken carcass samples. *Food Control*, 115, [107294]. <https://doi.org/10.1016/j.foodcont.2020.107294>

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Journal Pre-proof

The ability to detect campylobacter presence and concentration using different chicken carcass samples

Johanne Ellis-Iversen, Mette R. Gantzhorn, Birgitte Borck Høg, Alessandro Foddai, Maarten Nauta

PII: S0956-7135(20)30210-3

DOI: <https://doi.org/10.1016/j.foodcont.2020.107294>

Reference: JFCO 107294

To appear in: *Food Control*

Received Date: 17 January 2020

Revised Date: 30 March 2020

Accepted Date: 1 April 2020

Please cite this article as: Ellis-Iversen J., Gantzhorn M.R., Høg B.B., Foddai A. & Nauta M., The ability to detect campylobacter presence and concentration using different chicken carcass samples, *Food Control* (2020), doi: <https://doi.org/10.1016/j.foodcont.2020.107294>.

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2020 Published by Elsevier Ltd.

Credit author statement

I am unsure what is meant by this and have not been able to find information on line via Elsevier. Please could you advice on what this statement entails or guide me to template?

With thanks

Johanne Ellis-Iversen

The ability to detect *Campylobacter* presence and concentration using
different chicken carcass samples

Johanne Ellis-Iversen^{a,*}, Mette R. Gantzhorn^b, Birgitte Borck Høg^a, Alessandro Foddai^a, Maarten Nauta^a

Affiliations:

a) National Food Institute, Technical University of Denmark, 2800 Kgs. Lyngby, Denmark

b) Danish Veterinary and Food Administration, 2600 Glostrup, Denmark

*corresponding author:

Johanne Ellis-Iversen, National Food Institute, Technical University of Denmark, Kemitorvet, Building 201,
Room 27, 2800 Kgs. Lyngby, Email: joell@food.dtu.dk

Abstract

This study aimed to investigate the potential impact of using different sampling types, when testing broiler carcasses for *Campylobacter*. In Denmark, the current sampling type for surveillance is leg skin (LS) samples and since the EU process hygiene criterion uses pooled neck skin (NS) samples, it is relevant to understand the impact on surveillance outcomes the two sample types have.

Neck and skin leg samples were collected from 340 batches slaughtered at the two major broiler slaughterhouses in Denmark. A subset of 156 carcasses were allocated to generate 52 groups and five samples types were derived from each group : 1) single LS; 2) single NS, 3) mean LS, 4) mean NS and e) pooled NS (3 samples). All samples were enumerated for *Campylobacter* and were considered as positive if ≥ 10 colony forming units / gram (cfu/g). Prevalence, concentrations, agreement, association and epidemiological sensitivity, as the ability of the sample type to correctly classify a positive group, were compared between sample types. The variability when comparing a single LS to the pooled NS was explored using Monte Carlo analysis.

When comparing results on single LS and single NS from individual carcasses, the prevalence was 29.1% (CI_{95%} 24.3% ; 34.0%) and 34.7% (29.6%; 38.9%); respectively. The mean concentration of *Campylobacter* was 199 and 945 cfu/g; respectively. The cfu/g between the two sample types well correlated ($R^2_{adj} = 0.631$). However, the proportion of carcasses with >1000 cfu/g was significantly higher on NS (15.0%) than on LS (9.4%).

At group level, the prevalence derived from single LS samples was lower than all other sample types. Generally, the cfu/g on NS were higher than those on LS. For pooled NS the epidemiological sensitivity and prevalence were ≈ 1.6 times higher than in single LS, but still had moderate agreement (Kappa = 0.56, $p < 0.001$). The linear relationships between the cfu/g of different sample types was generally good (R^2 between 0.59 and 0.84). In the Monte Carlo analysis, association between the two sample types was found in 91.3% of the scenarios, suggesting some uncertainty in the ability to translate all outcomes between the two sampling types.

The study increases understanding of the impact of selecting a sample type for surveillance of *Campylobacter* in broilers. It could be expected that, by changing from single LS (current system) to the pooled NS, the estimated sensitivity and prevalence could both increase of ≈ 1.6 times.

Keywords: *Campylobacter*, broiler meat, carcass, leg skins, neck skins, microbiological criterion

Introduction

Campylobacter remains the pathogen causing most foodborne infections in the EU. In 2017, 250,161 human cases were reported in the EU and about 4,500 cases are reported annually in Denmark (EFSA and ECDC 2017). Due to the self-limiting nature of the disease, the number of reported cases is an underestimation and in Denmark a conversion factor of at least 12 is used to adjust for under-ascertainment (Pires et al, 2014).

The main food source of *Campylobacter* infection is poultry meat and around one third of all domestically acquired cases in Denmark are attributed to Danish chicken meat (Pires and Christensen, 2017; Kuhn et al., 2018). In 2008, the European Food Safety Agency (EFSA) carried out a union-wide baseline survey of *Campylobacter* in broiler batches at the end of the slaughter line (EFSA, 2010). It revealed high prevalence and high concentrations in most of EU, with large differences between countries. A quantitative microbiological risk assessment model estimated that, theoretically, the public health risk from poultry meat could be reduced by at least 50%, if all batches sold as fresh meat were contaminated with less than 1000 colonies forming unit/gram (cfu/g) of *Campylobacter* (EFSA, 2011, Nauta et al. 2012). A new microbiological process hygiene criterion (PHC) came into force in 2018 and is now mandatory for all member states in the EU (EU Commission, 2017). The PHC is measured by the presence and concentration of *Campylobacter* on pooled chicken neck skin sampled at the end of the slaughter line after chilling. The threshold for intervention is a specified number of samples with high level of contamination (>1000 cfu/g), starting at 20 of 50 samples in 2018 and dropping gradually to 10 of 50 samples in 2025. The statutory sampling for PHC has resulted in some Member States considering changing their existing *Campylobacter* surveillance sampling methodology to align with the new criterion (Duque et al., 2018, Reich et al., 2018). For other countries, this will be the first surveillance done for *Campylobacter* in broiler meat since the baseline survey.

As part of the National *Campylobacter* Action Plan in Denmark, the Danish Veterinary and Food Administration (DVFA) has carried out surveillance for *Campylobacter* in samples of chicken leg skins at broiler slaughterhouses since 2013 (one single leg sampled from approximately 1000 flocks per year). Action plans against *Campylobacter* have been in place since 2008 and the newest action plan commenced in 2018. The main goal of the plan is to reduce human *Campylobacter* cases by 5% annually, to maintain the low prevalence of *Campylobacter* positive broiler flocks and to continue reducing the relative risk of becoming ill from domestically produced broiler meat (DVFA, 2018).

To evaluate this target, a risk model is applied. The model calculates the risk of human exposure from poultry meat given the current presence and concentration of *Campylobacter* on chicken leg skin. This risk model is essentially the same as the one used by EFSA (2011) (Nauta et al., 2012), as it applies the same consumer phase model (Nauta et al., 2008) and dose response model (Teunis and Havelaar, 2000). The outcome of the model is a relative risk: the risk today versus the risk estimated in 2013.

To accommodate the EU PHC on *Campylobacter* in broiler carcasses, the DVFA considers changing the current sampling plan, and relying on the results of the neck skin samples to estimate the relative risk in the future. However, the estimates of the relative risk may change, if the surveillance samples change from single

leg skin (LS) samples to pooled neck skin (NS) samples and the obtained data are applied in the same risk model. To allow the use of the historic surveillance data for comparative analyses, it is important to understand the association between the outcomes from the different sample types, i.e. between single LS samples and the pooled NS samples taken per batch. Therefore, this study aims to assess the difference between results from different sample types, if the sample type changes in the future.

1. Materials and methods

1.1. Samples

A total of 340 carcasses from 340 different flocks were selected at the two main chicken slaughterhouses in Denmark between 29th of May to 29th of September 2017. A systematic sampling approach was applied and the samples were stratified on time. From each carcass, the neck skin and either one or two legs were collected after cooling and were packed individually. The samples were stored between 1-8°C for a maximum of 24 hours before transport to the laboratory.

At the laboratory, the legs were skinned and a minimum 10 grams of leg skin (LS) was used for analysis. Each neck skin (NS) was divided into two pieces, one (approx. 3 grams) was analysed directly for *Campylobacter* and one was used for pooling (3.5 grams) together with neck skins from two other chickens. Furthermore, a subset of 156 carcasses were allocated to 52 groups of three carcasses each.

1.2. Laboratory methods

Direct plating according to standard NMKL 119, 3 Ed. 2007 was performed to quantify *Campylobacter* in all sample types. The dilution range was extended to 10⁻¹ cfu/g yielding a minimum detection level of 10⁻¹ cfu/g.

1.3. Data analyses

The microbiological enumeration method had a detection limit of 10 cfu/g and plates without growth were registered as <10 cfu/g. For analytical purposes, samples <10 cfu/g were considered negative and otherwise positive. From carcasses, where two LS were analysed, the mean of the two results was used, to represent the leg skin concentration for the bird. Results from the same birds were compared using linear regression on log-transformed cfu/g to describe the linear relationship and correlation was assessed by Pearson coefficients.

1.4. Comparison of results from five different sample types in the individual carcasses and in the 52 groups

Firstly, five sample types for each of the 52 groups were created as: a) random single LS, b) mean LS, c) single NS, d) mean NS, and e) pooled NS (Figure 1). Secondly, an additional sample type (stochastic LS) was created later in the analysis to investigate uncertainty when relating sampling types.

The random single LS per group was determined by randomly selecting (in Excel), one of the three leg skins per group and disregarding the others.

The data was described for each sample type both as binary and continuous variables. Thereafter, the prevalence and concentrations of *Campylobacter* at both carcass and group levels were compared.

The mean concentration (cfu/g) of the three single neck skins in the group was used to calculate the variable: 'mean NS'. Similarly, the mean cfu/g from the three individual legs (LS) skins from the three birds in the group was used for the variable: 'mean LS'.

To understand the linear association between concentrations of *Campylobacter* in the different sample materials linear regression analyses and Pearsons coefficient were used. The concentration of *Campylobacter* in the different sample types was log-transformed for normalization before comparison.

The epidemiological group level sensitivity was defined as "The proportion of positive groups classified by the sample type out of the truly positive groups" (Akobeng 2007; Lewis *et al*, 2012). This was calculated for each sample type by comparison to a 'gold standard' classification of each group of carcasses. The gold standard was derived by classifying a group as positive, if at least one of the five sample types were positive and thus, the group was considered truly positive (Lewis *et al*, 2012).

The agreement in ability of a sample type to classify the group of carcasses between the results from each sample type was assessed calculating the kappa statistics and the overall agreement (in %). The kappa statistic and the strength of agreement were translated into qualitative terms, by using the scale proposed by Landis and Koch (1977).

All statistical analyses were carried out using STATA 14 (STATACorp, Texas).

1.5. Monte Carlo analysis to investigate uncertainty due to sampling design

Monte Carlo analysis was carried out to investigate whether the association between: i) the randomly selected single LS and ii) the pooled NS was dependent upon, and on which of, the three LS (from each group) drawn in the selection.

Furthermore, the effect of the uncertainty caused by the variation present between different selections of single LS was investigated. Different sets of 52 single LS were analysed by: a) sampling in a Monte Carlo simulation one of the three single LS results per group and b) creating 100.000 combinations of single LS sample results for the 52 groups.

The association between the two sample types was studied and the regression lines produced from the Monte Carlo simulation were compared. The Monte Carlo simulation was done in @Risk (version 6.2, Palisade) an add-on to Excel.

2. Results

2.1. Prevalence, concentration and association of *Campylobacter* on the same carcasses

A total of 400 leg skins from 340 carcasses were sampled. Thus, from 60/340 carcasses, both legs were collected and 3/60 carcasses (5%) showed disagreement in terms of presence/absence; because one leg from the bird was positive and the other leg was negative. In all three disagreeing carcasses, the positive leg skin had a very low concentration of *Campylobacter* (10 cfu/g). Moreover, the vast majority of two-leg sampled carcasses (82.5%) had similar concentrations (within 1 log) on both legs, while the remaining percentage showed differences between legs, which ranged from 10 and 3900 cfu/g.

When considering all 340 carcasses and using only one randomly selected LS from the 60 carcasses from which two legs were sampled, the prevalence of positive LS was 29.1% (CI_{95%}: 24.3; 34.0). The prevalence of positive single NS was 34.7% (CI_{95%}: 29.6; 39.8) (Table 1) yielding no significant difference between the two sample types.

The concentrations on single LS and NS samples are summarized in Table 1. A total of 9.41% of the legs were above the threshold of 1000 cfu/g, relevant for the EU hygiene criteria. The proportion of NS above the EU process hygiene criteria threshold was significantly higher than the proportion of LS (15.0 % versus 9.4%, $p = 0.03$).

The association between log cfu/g in both individual sample types from the same positive carcasses was good (regression line $y=0.867x + 0.761$, $p>0.01$, $R^2_{adj}= 0.631$) and the correlation was strong (Pearson= 0.7873) (Figure 2).

2.2. Prevalence of positive groups and concentration per group across five different sample types

The chicken were collated in groups of three and five different sample types (mean NS, mean LS, random single LS, stochastic single LS, pooled NS) within the 52 groups were compared according to prevalence and concentration of *Campylobacter* (Table, 2).

The lowest prevalence was found using random single LS (36.5%) and stochastic single LS (35.3%); while the highest prevalence was found using mean NS (65.4%). Thus, the prevalence of positive groups was ≈ 1.6 times higher with the pooled NS (59.6%) than using the single LS (Table, 2).

Campylobacter cfu/g were generally higher in NS than in LS. All five sample-types found less than 25% of groups above the EU hygiene criterion cut-off (>1000 cfu/g), but the range of concentrations within each sample type was wide. The single random and the single stochastic LS samples classified only 8% and 10% (respectively) of the groups as above the EU hygiene criterion cut-off, whereas the mean and the pooled NS samples classified 23% and 19% of the groups above the cut-off (Table 2).

2.3. Agreement between sample types across groups and epidemiological sensitivity of the sample type

The agreement between different sample types was generally good and only five groups were classified differently as negative or positive, by the mean NS and mean LS samples. Four of these were positive by mean NS, but negative in the mean LS samples; while one sample was negative by mean NS and positive by mean LS. Overall, the two sampling types had a substantial agreement of 90.28% and a statistically significant Kappa value of 0.796 ($p < 0.001$). Moreover, all five discordant samples had low concentrations of *Campylobacter* in the positive sample (< 40 cfu/g).

The agreement in ability of a sample type to classify the group of carcasses between mean NS and pooled NS samples was almost perfect at 94.23% (Kappa=0.88, $p < 0.001$) with only three discordant groups which were positive in mean NS, but negative in pools. The concentrations of *Campylobacter* in all three discordant sample types were low (< 40 cfu/g).

The best agreement in ability of a sample type to classify the group of carcasses was found between mean LS samples and pooled NS samples with an almost perfect agreement of 96.15% (kappa = 0.92, $p \leq 0.001$). In the two discordant groups, one was positive only by the mean LS, while the other was positive only by the pooled NS. The disagreeing groups had very low concentrations at 10 cfu/g and 30 cfu/g, respectively.

The agreement in ability of a sample type to classify the group of carcasses between the single LS and the pooled NS was moderate (76.9%, Kappa=0.56, $p < 0.001$). In 12 groups, the pooled NS were positive where the single LS skin was negative, but all other samples classifications were in agreement. In the ones that disagreed, the cfu/g varied from 10 to 4200 in the NS pools, with a mean of 773 cfu/g *Campylobacter*.

2.4. Comparison of epidemiological sensitivity using different sample types

A total of 35 groups (67.3%) were positive for *Campylobacter* by at least one sampling type. Considering this as the gold standard, the sensitivity of both the mean LS samples and the pooled NS samples being positive was 88.6%. This means that both sample types were able to correctly classify 88.6% of all positive groups as positive. The sensitivity of the mean NS and randomly single LS were 97.7% and 54.3%, respectively. The ratio between the sensitivity estimated using the pooled NS and the random single LS was $88.6\% / 54.3\% \approx 1.6$.

2.5. Associations and correlations between concentrations of *Campylobacter* in different sample types

A clear linear relationship in concentrations was found between paired sample types for positive groups (Table 3 and Figure 3). A good linear relationship ($R^2 = 0.587$, $p < 0.01$) and moderate correlation (Pearson correlation coefficient = 0.6317) was found between the log cfu/g on positive random single LS and pooled NS (Table 3, Fig. 3B).

Stochastic selection of single LS yielded some variation in regression lines, intercept and R^2 values (figure 4 and table 4). The majority of the regression lines (91.3%) were significantly different from 1 suggesting that an association between the concentrations on single LS and pooled NS was usually but not always present.

3. Discussion

The passing of Regulation (EC) No 2017/1495, amending Regulation (EC) No 2073/2005 as regards *Campylobacter* in broiler carcasses is likely to prompt review of current surveillance and sampling practices at the slaughterhouses in the different member states (Duque 2018, Reich 2018). This provides an opportunity to consider changing current surveillance practices and using the outputs from the PHC sampling instead. In Denmark, this would entail changing from public sampling and surveillance to industry-driven surveillance and private laboratory analyses and could provide a saving to public funding, if the sampling schemes, laboratories etc. are equally sensitive and reliable (DVFA, 2020).

Changing of sampling methods in surveillance programmes may lead to changes in outcomes and trends, which may not reflect the true trend in pathogen occurrence. Understanding the magnitude of expected changes and the influence on trends in advance will support risk management during and after the changes. The work in the paper is relevant to all countries considering whether to change methods to comply with the EU hygiene criterion.

The prevalence of positive groups was higher, when three samples from a group were analysed compared to only analysing a single leg skin (LS). This is due to a higher probability of obtaining at least one positive result, when three samples are analysed rather than just one. The fact that the prevalence doubled suggests that the epidemiological sensitivity of single leg sampling is quite low and this should be kept in mind, when changing the surveillance programme. It is very likely that the estimated prevalence of positive batches/flocks will increase, when the sampling method increases in sensitivity.

In fact, the agreement between the current sampling protocol (single LS) and the alternative EU sampling (pooled NS) appeared to be “moderate”. With pooled NS samples, the percentage of groups classified as above the EU hygiene criterion cut-off was approximately 2.4 times higher than with the single LS sample type (Table 2).

Moreover, the ratio between the sensitivity estimated using the pooled NS and the sensitivity estimated using the single LS was ≈ 1.6 . A similar ratio was found between the apparent group prevalence estimated with the

two systems (Table 2). It is likely that by changing from single LS to the new EU samples of pooled NS, the prevalence may increase by ≈ 1.6 .

Campylobacter prevalence and concentration are very dependent on sampling site on the chicken. In general, slightly higher concentrations of *Campylobacter* were found on neck skins compared to leg skins. Similar results were seen in France (Duque, 2017). It is expected that neck skins have higher concentrations than leg skins due to the chicken hanging upside down on the slaughter line causing run off including bacteria from the whole carcass to gather in the neck skin. Furthermore, the neck skins are more exposed to external contamination from its physical position on the slaughter line. It may drag along equipment or swing and touch neighbouring birds. The lower concentration of *Campylobacter* in pooled neck skins as opposed the individual necks skins in the pool is likely caused by a lower sensitivity of pooled analysis.

The observed variation in concentrations between skin samples within flocks is of interest, as the EFSA baseline study (EFSA, 2010) sampled only one skin sample per flock and it was assumed that the result of this single sample was representative for the whole flock. Our analyses show that the use of single samples may result in an underestimation of the true prevalence, especially if the detection limit is high in relation to the observed concentrations. For example, Hansson et al (2010) used an alternative enumeration method (carcass rinsing) and found a considerable variation in concentrations within flocks, but the presence/absence results hardly varied within flocks. In all comparisons, the percentage of samples > 1000 cfu/g was higher with neck skins than with leg skin (Table 1 and 2). Nevertheless our results also show that finding a concentration higher than 1000 cfu/g is not necessarily representative for the whole flock.

Despite the fact that pools of neck skins are less sensitive due to analytical methods applied in the laboratory and leg skins are less likely to be contaminated than neck skin samples due to industrial processing conditions, a very good fit was identified between the results for mean leg skins (mean LS) and for pooled neck skins (mean NS). The classifications by these sample types were more similar than any of these compared to the individual NS or single LS. This is probably because both sample types include more samples, thus reducing the variation.

The large difference in *Campylobacter* concentrations between using the mean cfu/g from three birds and the results from one random leg may be due to several factors. Initially, the mean of three legs will be positive, if one of the three legs was positive, increasing the probability of the group being positive. Sampling only one leg from the group resulted in a much lower concentration and a lower probability of the group being positive.

The differences between the single LS and the pooled NS could also be explained by the lower sensitivity of only sampling one bird instead of three, even if the three are pooled. This also influenced the association between the two samples types, which were slightly less correlated than all the other sample types (Table, 4).

Furthermore, the cfu/g for LS and NS were only correlated in 91% of the times. This is relevant to keep in mind, when considering the implementation of the new EU process hygiene criterion, or when translating

concentrations on single LS into concentrations found in pooled NS. Nonetheless, the study still provides sufficient understanding to translate surveillance prevalence and concentrations on LS to the future process hygiene criteria based on pooled NS and vice versa. If major changes in production, processes, or campylobacter epidemiology occur, we recommend repeating the study. We also recommend validating the translation estimates on actual surveillance data over the next year to increase accuracy by increasing the sample size, and to evaluate if there are seasonal differences in the relation between leg and pooled neck skin.

4. Conclusions

The potential impact of changing samples from legs skin to neck skin for *Campylobacter* surveillance on chicken carcasses was investigated, to consider whether the EU process hygiene criteria samples could replace the current surveillance in Denmark. The mean concentration of *Campylobacter* was higher in single necks skin (NS) samples than in single leg skin (LS) samples of the same chicken, but the difference was fairly consistent and the two single sample types showed a good association.

When the two sample types were used to classify groups of three carcasses, a “moderate” agreement was found between using one single leg skin and three pooled neck skin samples per tested group/flock. The pooled NS were more sensitive and increased the prevalence by 1.6 times compared to the single LS. The pooled NS samples also identified more groups above the EU hygiene criterion cut-off (> 1000 cfu/g).

The challenge of selecting one out of the three single leg skins within the group was handled by using Monte Carlo simulations. A significant association between the concentrations on a single LS and the pooled NS within a group was found in more than 90% of the simulations, suggesting that an association between the sample types is highly likely.

The results of this study cannot be directly extrapolated to the situation in the national surveillance system. However, it provides confidence that the sample types are correlated today. However, if major changes in production, processes, or campylobacter epidemiology occur, we recommend repeating the study. We also recommend validating the translation estimates on actual surveillance data over the next year to increase accuracy by increasing the sample size, and to evaluate if there are seasonal differences in the relation between leg and pooled neck skin.

Acknowledgement

This survey was funded by the Danish Veterinary and Food Administration, who also sampled and carried out the laboratory analysis.

Conflict of interest

Declarations of interest: none

5. References

- Akobeng, A.K. (2007), Understanding diagnostic tests 1: sensitivity, specificity and predictive values. *Acta Pædiatrica*, 96: 338-341. doi:[10.1111/j.1651-2227.2006.00180.x](https://doi.org/10.1111/j.1651-2227.2006.00180.x)
- Anonymous, 2007. Thermotolerant *Campylobacter*. Detection, semi-quantitative and quantitative determination in foods and drinking water, Method No. 119, 3. Ed. Nordic Committee on Food Analysis, Helsinki, Finland.
- DVFA, 2020. Danish Veterinary and Food Administration, 2018. Handlingsplan for campylobacter i slagtekyllinger, fødevarer og det omgivende miljø, 2018-2021
<https://www.foedevarestyrelsen.dk/Kontrol/Kontrolresultater/Sider/Overvågning-og-kontrol-for-campylobacter-i-fødevarer.aspx> Accessed 29/02/2020
- Duqué, B., Daviaud, S., Guillou, S., Haddad, N., Membré, J.-M., 2018. Quantification of *Campylobacter jejuni* contamination on chicken carcasses in France Food Research International 106 (2018) 1077–1085
- EFSA, 2010. Analysis of the baseline survey on the prevalence of *Campylobacter* in broiler batches and of *Campylobacter* and *Salmonella* on broiler carcasses in the EU, 2008, Part A: *Campylobacter* and *Salmonella* prevalence estimates. *EFSA Journal* 2010;8(03):1503. [100 pp.]. doi: 10.2903/j.efsa.2010.1503
- EFSA, 2011, EFSA Panel on Biological Hazards (BIOHAZ); Scientific Opinion on *Campylobacter* in broiler meat production: control options and performance objectives and/or targets at different stages of the food chain. *EFSA Journal* 2011;9(4):2105. [141 pp.]. doi:[10.2903/j.efsa.2011.2105](https://doi.org/10.2903/j.efsa.2011.2105).
- EFSA & ECDC, 2017, EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2016. *EFSA Journal* 2017;15(12):5077
- EU commission, 2017, Regulation (EC) No 2017/1495, amending Regulation (EC) No 2073/2005 as regards *Campylobacter* in broiler carcasses https://www.fsai.ie/uploadedFiles/Reg2017_1495.pdf
- Hansson, I., Pudas, N., Harbom, B., Olsson Engvall, E., 2010. Within-flock variations of *Campylobacter* loads in caeca and on carcasses from broilers. *International Journal of Food Microbiology* 141, 51–55.
- Kuhn, K.G., Nielsen, E.M., Mølbak, K., Ethelberg, S., 2018 Determinants of sporadic *Campylobacter* infections in Denmark: a nationwide case-control study among children and young adults *Clinical Epidemiology* Volume 2018:10 Pages 1695—1707

- Lewis FI, Torgerson PR. A tutorial in estimating the prevalence of disease in humans and animals in the absence of a gold standard diagnostic. *Emerg Themes Epidemiol.* 2012 Dec 28;9(1):9. doi: 10.1186/1742-7622-9-9. PMID: 23270542; PMCID: PMC3558341.
- Landis, J.R., Koch, G.G., 1977. The measurement of observer agreement for categorical data. *Biometrics* 33: 159-174.
- Nauta, M.J, Sanaa, M., Havelaar, A.H., 2012. Risk based microbiological criteria for *Campylobacter* in broiler meat in the European Union. *International Journal of Food Microbiology* 158:209-217.
- Nauta, M.J., Fischer, A.R.H., Van Asselt, E.D., De Jong, A.E.I, Frewer, L.J. and De Jonge, R., 2008. Food Safety in the Domestic Environment: the effect of consumer risk information on human disease risks. *Risk Analysis*, 28, 179-192.
- Pires, S., 2014. Burden of Disease of Foodborne Pathogens in Denmark, Technical Report. ISBN: 978-87-93109-31-5. <http://www.food.dtu.dk/english/-/media/Institutter/Foedevareinstituttet/Publikationer/Pub-2014/Burden-of-Disease-of-Foodborne-Pathogens-in-Denmark.ashx?la=da&hash=D82FE4026BB3DE49C3B2EEA7F535011175882203>
- Pires, S.M., Christensen, J., 2017, Source attribution of *Campylobacter* infections in Denmark - Technical Report. National Food Institute, Technical University of Denmark, Kgs. Lyngby
- Reich, F. Valero, A., Schill, F., Bungenstock, L., Klein, G., 2018. Characterisation of *Campylobacter* contamination in broilers and assessment of microbiological criteria for the pathogen in broiler slaughterhouses, *Food Control*, Volume 87, 60-69, <https://doi.org/10.1016/j.foodcont.2017.12.013>.
- Rosenquist, H., Bengtsson, A., Hansen, T.B., 2007. A collaborative study on a Nordic standard protocol for detection and enumeration of thermotolerant *Campylobacter* in food (NMKL, 3. Ed., 2007). *Prev. Vet. Med.* 2007; 118; 201-213
- Teunis, P. F., and Havelaar, A. H., 2000. The beta Poisson dose response model is not a single-hit model. *Risk Analysis*, 20(4), 5, 13–520

1 **Figure 1.** Sampling strategy and sample type generation from chicken carcasses to test the ability of surveillance different sample types.

2
3 **Figure 2.** The correlation between *Campylobacter* cfu/g (log) in single leg skin (LS) and in single neck skin (NS) from 99 positive chicken
4 carcasses.

5
6 **Figure 3.** The relationship between concentrations of *Campylobacter* in 52 broiler groups measured on mean leg skin (LS) and pooled neck skin
7 (NS) (**A**) and on single LS and pooled NS (example of one set of randomly selected LS (**B**)).

8
9 **Figure 4.** Graph showing 50 randomly obtained linear regression lines found by Monte Carlo simulation by randomly selecting one of three leg
10 skins per group and comparing the concentration of *Campylobacter* in the sample to the pooled neck skins.

11

Table 1. The concentration (cfu/g) of *Campylobacter* in single leg skins (LS) and single neck skins (NS) from 340 slaughter chickens.

		N	Mean	Minimum	Maximum	> 1000
Single LS	cfu/g	340	299	0.0	7500	9.4 %
	Log cfu/g	99	2.45	0.7	3.88	
	Prevalence	29.1%				
Single NS	cfu/g	340	945	0.0	21000	15.0 %
	Log cfu/g	118	2.61	1.0	4.32	
	Prevalence	34.7%				

Table 2. *Campylobacter* detection and concentration in 52 groups using four different sample types. LS = Leg skin; NS = Neck skin.

	% positive groups	Mean cfu/g	Mean log cfu/g	Log of mean cfu/g	>1000 cfu/g (%)	P50 cfu/g	P75 cfu/g	Max. cfu/g
Mean NS	65.38 [52.01; 78.76]	1116	2.65	3.05	12(23)	230	883	14000
Pooled NS	59.62 [45.82; 73.41]	886	2.66	2.95	10(19)	145	800	20000
Mean LS	59.62 [45.82; 73.41]	350	2.26	2.54	6 (12)	22	435	4683
Random single LS	36.5 [23.0; 50.1]	276	2.44	2.32	4 (8)	0	108	5150
Stochastic single LS ¹	35.26 [26.9; 44.2]	350 [207; 551]	2.49 [2.23;2.77]	2.53 [2.32;2.71]	5 (10) [2;8(4;15)]	0 [0;0]	123.3 [15;320]	5088 [4000;5900]

¹ Obtained by Monte Carlo simulation; mean of 100,000 iterations and [2.5 ; 97.5 percentile]**Table 3.** Regression analyses of the association between the concentrations of *Campylobacter* on groups found positive by using different sample types. LS = Leg skin; NS = Neck skin.

	No. groups with >1 log Difference	Linear Regression Coefficient	CI 95%	Constant	Observations in model	p-value	Adjusted R ²
Single NS vs. pooled NS	6	0.801	0.673; 0.929	0.427	31	<0.001	0.844
Single LS vs. pooled NS	23	0.632	0.222; 1.044	0.673	19	0.005	0.587
Mean LS vs. mean NS	25	0.835	0.576; 1.095	0.898	30	<0.001	0.594
Mean LS vs. pooled NS	24	0.758	0.549; 0.968	0.943	30	<0.001	0.650

Table 4. Regression analyses of association of the concentrations of *Campylobacter* on three chicken carcasses measured by Monte Carlo simulation-selected single LS samples vs. pooled NS samples.

Single leg skin (LS)	Linear Regression Coefficient (CI 95%)	Constant	p-value	Observations in model	Adjusted R ²
Randomly selected	0.632 (0.22;1.04)	0.673	0.005	19	0.587
Stochastic ¹	0.608 (0.28; 0.99)	1.306 [0.205; 2.24]		18.3 [14; 23]	0.351 [0.10; 0.62]

¹ Results of Monte Carlo simulations, mean of 100,000 iterations; [2.5; 97.5 percentile]

Figure 3.

Figure 4.

Figure 1.

Figure 2

Highlights

- Detection of *Campylobacter* from legs and neck skins of Danish broilers was compared
- Neck skins were equally or more sensitive than legs for detecting *Campylobacter*
- Agreement was moderate-almost perfect for different combinations of sample types
- Good linear association in cfu/g was found between most sample types
- Monte Carlo Analysis showed the uncertainty translating results across sample types