Impact of post-slaughter broiler meat treatments on human disease risk from *Campylobacter*-contaminated meals

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SUMMARY

Campylobacter is a major cause of foodborne gastroenteritis worldwide, with poultry meat as the primary human exposure route. This study quantified how two post-slaughter meat treatments—slurry-ice carcass cooling and high-oxygen modified-atmosphere packaging (MAP-O_2 with ≈ 80% O_2 and 20% CO_2)— can affect human campylobacteriosis risk from broiler meals in Denmark. Here, MAP-O_2 is commonly used to extend product´s shelf life, whereas slurry-ice is not yet applied extensively, but appears promising for faster carcass chilling. Recent field studies indicated that both treatments reduce *Campylobacter* concentrations (colony forming units per gram, cfu/g). We used 2024 Danish surveillance data from four slaughterhouses (SA–SD), consisting of leg-skin (LS) concentrations from 1,296 flocks. These data were integrated into the quantitative microbial risk assessment (QMRA) model from the Danish Action Plan for *Campylobacter*. Human average risk per meal was estimated for both monthly and annual surveillance periods. Next, the relative risk (RR) was defined as the ratio between scenario-specific risk estimates and the baseline mean risk from 2019–2021. Six scenarios were evaluated: a reference scenario (without considering any meat treatment) and five mitigation scenarios using slurry ice, MAP-O_2, or their combination. Treatment-specific reductions in concentrations, when changing from the reference to a mitigation scenario, were taken from recent Danish research. Next, two Generalized Estimating Equation (GEE) models, were used to assess statistically significant effects on concentrations and related apparent flock prevalence (AP), which affected the risk estimates. Slurry-ice produced a 7% reduction in the overall annual RR, while MAP-O_2 caused much larger effects across seasons and slaughterhouses, lowering the annual RR by 67–84%. GEE analyses confirmed significant pairwise interactions of season, slaughterhouse, and scenario, with MAP-O_2 giving the strongest AP and concentration reductions during summer, especially at higher-risk slaughterhouses. Overall, MAP-O_2 emerges as an effective post-slaughter risk mitigation measure.

1. INTRODUCTION

Campylobacter spp. infections remain a major cause of foodborne bacterial gastroenteritis worldwide. Poultry meat is consistently identified as the leading source of consumers exposure, largely due to contamination during slaughter and processing [1]. Faecal leakage and cross-contamination (e.g. through contaminated equipment) can spread the pathogen onto carcasses. Although chilling can reduce bacterial survival, *Campylobacter* may persist on fresh poultry products and be transferred to consumers through improper handling and/or insufficient cooking.

Denmark operates a comprehensive surveillance and risk assessment system under the National Action Plan for *Campylobacter* [2]. Central to this system is a quantitative microbial risk assessment (QMRA) model from Nauta et al. [3], which integrates flock prevalence, carcass contamination, consumer handling, and dose–response modelling. Previous Danish work using this framework has shown that flock prevalence varies remarkably across farms, slaughterhouses, and seasons [2]. Summer months consistently display higher flock prevalence and human incidence. Within this context, recent Danish research [4, 5] investigated effects of post-slaughter meat treatments—i.e. those applied before retail—on *Campylobacter* meat concentrations. Those treatments were:

a) Slurry-ice spraying [5]. Currently, not systematically applied in Danish slaughterhouses.
b) Modified atmosphere packaging (MAP), with high-oxygen concentrations (i.e. MAP-O_2 with ≈ 80% O_2 and 20% CO_2) which is already widely implemented in Denmark for retailed poultry meat [4].

The aim of this study was to assess the effects of slurry-ice and MAP-O_2 on the risk of human campylobacteriosis posed by a *Campylobacter*-contaminated broiler meal, according to the new science-based information from Calvo-Fernandez et al. [4, 5].

2. MATERIALS AND METHODS

2.1 Surveillance data

Surveillance data from 2024 were obtained from the Danish Veterinary and Food Administration. The dataset included 1,330 leg skin (LS) samples (one sample per flock) collected at the four main Danish slaughterhouses referred here as: SA, SB, SC, and SD. After data handling (i.e., keeping formatted flock-ID identifiers), 1,296 flocks were maintained. Metadata included: slaughter date, flock-ID, flock type (organic or conventional), farm-ID (CHR), slaughterhouse, and culture results (in colony forming units per gram, cfu/g). Apparent flock prevalence (AP, not corrected for test performance) was defined as the proportion of flocks with contaminated LS samples ≥10 cfu/g. Data handling, descriptive statistics and statistical analysis were carried out in the free software R (version 4.5.2).

2.2 The QMRA model

The QMRA model estimated average monthly and annual probabilities of human illness from a broiler-based meal. Next, the relative risk (RR) was estimated, to compare the 2024 (monthly and annual level) risk per serving against the average risk from the three-years period 2019–2021, representing the baseline risk within the Action Plan. The model preserves consumer behaviour and dose-response variability constant across scenarios and surveillance periods, ensuring that RR changes stem from scenario-specific surveillance inputs (i.e. data-driven) [2].

2.3 Risk-mitigation scenarios

Six risk assessment scenarios were investigated: a reference (ScenRef = no mitigation) and five mitigation scenarios applying slurry-ice, MAP-O_2 or both, based on different concentrations reduction factors. Accordingly, six scenario data tables were created (each including the 1,296 flocks with adapted concentrations) and fed independently into the QMRA model. Percentage (relative) reduction factors for *Campylobacter* concentrations (used to create the different scenarios), were set considering different meat cuts:

ScenI (slurry-ice on LS): 10.9% cfu/g reduction [5]
ScenII (MAP-O_2 on neck skin): 84.5% [4]
ScenIII (MAP-O_2 on breast fillet): 94.2% [4]
ScenIV (average of ScenII and ScenIII): 90.6%
ScenV (slurry ice + MAP-O_2): i.e. ScenIV reduction applied on values from ScenI

Descriptive statistics on APs and meat concentrations distributions (across positive flocks) were carried out for each scenario, quarter of the year (Q1-Q4) and slaughterhouse.

2.4 Statistical analysis

For the statistical analysis the six scenario tables used in the QMRA model, were combined into an overall analytical dataset. Next, two Generalized Estimating Equation (GEE) models were fitted: i) a binary (logistic) GEE model considering response variable: flock result = positive or negative (1,296 * 6 = 7,776 records), and 2) a linear (Gaussian) GEE model with response variable: log10(cfu/g) across positive flocks (1,856 records).
In both models, predictors included: scenario, quarter, slaughterhouse, and all their pairwise interactions. Robust standard errors and Tukey-adjusted post-hoc comparisons were used. Cluster structure was defined by flock-ID. Assumptions of statistical models were evaluated. Sensitivity analysis was also carried out by: a) comparing the Quasi-Likelihood Information Criterion (QIC) across different working-correlation structures within each GEE model, and b) investigating alternative mixed-effect models with farm-ID or within-farm stable unit variables set as random factors.

3. RESULTS

3.1 QMRA outputs

Under the 2024 reference scenario (ScenRef), the QMRA estimated an annual relative risk (RR) of 1.03, indicating a slight (+ 3%) increase of risk per meal, compared with the 2019–2021 baseline estimate. Applying slurry ice (ScenI) reduced the annual RR marginally to 0.96 (i.e. -7%). In contrast, MAP-O_2 scenarios generated substantially larger reductions (67-84%): ScenII yielded an RR of 0.34, ScenIII an RR of 0.17, and ScenIV an RR of 0.24. Combining slurry-ice with MAP-O_2 (ScenV, RR = 0.22) provided a minor improvement over MAP-O_2 alone (ScenIV). Moreover, MAP-O_2 scenarios appeared capable of maintaining monthly RRs consistently below 1.0 across all months.

3.2 Outputs of descriptive statistics

In ScenRef, 418 of the 1,296 flocks tested positive (AP = 32.3%). Mitigation scenarios reduced this overall annual AP to 29.2% (ScenI), 22.8% (ScenII), 18.2% (ScenIII), 20.5% (ScenIV), and 20.3% (ScenV). In general,

AP showed strong seasonal variation, with Q3 exhibiting the highest values (58.5%). Slaughterhouse SC showed the highest AP (64.9%), followed by SD (49.5%), SA (26.0%) and SB (18.7%). Organic flocks showed higher AP than conventional flocks (49.5% vs. 30.9%).
Carcass *Campylobacter* concentration across positive flocks, was higher in the ScenRef and lowered across scenarios, with seasonal (Q1 > Q2 > Q3 > Q4), slaughterhouse (SC > SB > SA > SD), and flock-type (conventional > organic) patterns.

3.3 Outputs of statistical analysis

GEE-based odds ratio (OR) estimates (Figure 1) confirmed that ScenRef consistently had the highest odds of carcass-flock positivity across all slaughterhouses and quarters. All pairwise interactions were significant. Slurry-ice (ScenI) resulted in modest odds reductions compared to ScenRef, while MAP-O_2 based scenarios produced large decreases. The strongest effects occurred in Q3, in all slaughterhouses, under ScenIII. Between slaughterhouses (in the same season and scenario) ORs were higher at SD (mean OR = 10.3), followed by SA (3.3), SC (2.8) and SB (2.4) (Figure 1).
The linear GEE model showed that concentration levels were strongly influenced by scenario, with ScenRef and ScenI yielding the highest predicted log10 concentrations across all quarters and slaughterhouses, with lower values under ScenII to ScenV. These reductions, however, varied substantially by season and slaughterhouse, according to the significant (pairwise) interaction terms. The largest scenario-driven decline in concentration occurred at SC in Q2, where MAP-O_2 reduced the predicted mean log10 cfu/g by up to −1.02 (decrease from 2.67 in ScenRef to 1.65 in ScenIII).
Diagnostic checks confirmed stability of the two GEE models estimations, realistic predicted probabilities (logistic model) and concentrations (linear model), and absence of problematic collinearity. The independence working-correlation structure provided the best (lowest) QIC fit for both GEE models. Mixed-effects models showed fitting issues, confirming the GEE approach as preferable for the aimed flock population-average inference.

4. DISCUSSION

4.1 Main findings

This study quantitatively showed that MAP-O_2 can be a highly effective post-slaughter mitigation measure for reducing *Campylobacter* concentrations, prevalence, and related human risk from broiler-based meals. It consistently reduced apparent prevalence and carcass contamination across all seasons and slaughterhouses, causing 67–84% reductions in the annual RR per broiler-based serving. MAP-O_2 is already widely applied in Denmark, meaning that, apart its benefits on prolonged product´s shelf life, there is a relevant concomitant *Campylobacter* risk mitigation.
For slurry-ice spraying, more modest risk reductions were estimated. It is not currently implemented systematically in Danish slaughterhouses, but it appeared that this measure alone would not substantially reduce public-health risk, though it may still provide marginal supplementary benefits if combined with other measures, as shown in ScenV. Moreover, it should not be excluded that in the future, greater reductions in *Campylobacter* concentrations may be achievable through slurry-ice spraying, as research continues and the specific biological mechanisms of action on *Campylobacter* through this technology, become clearer. Such a measure has been developed mainly to speed up the chilling process and to increase production capacity, i.e. to reduce the slaughter line energy costs and to optimize productivity.
The GEE models corroborated the descriptive statistic results and their related QMRA findings. Both models showed that scenario effects on prevalence and contaminations varied across quarters and slaughterhouses and were relevant especially under MAP-O_2 treatment. The largest prevalence reductions occurred during Q3 (when *Campylobacter* prevalence typically peaks in Denmark), and at the highest-risk slaughterhouses. Thus, MAP-O_2 optimization—ensuring consistent high-oxygen conditions across all product lines and flocks, particularly during summer—can yield significant overall risk-mitigation benefits.
Overall, in this study, it is shown that, integrating post-slaughter treatments with upstream interventions could produce additive risk mitigations effects along the food chain. Several mitigation measures have already been investigated in different studies, such: farm-level high-biosecurity, logistic-slaughter strategies, hygiene improvements at slaughterhouses (to reduce cross-contaminations) and use of feed and water additives. The effect of feed and water additives was recently investigated in Denmark, but was not included here, because the caecal *Campylobacter* presence was measured; but not the meat concentrations which are needed to inform the QMRA model. The Danish Action Plan already emphasizes risk-based and surveillance data-driven approaches for decision-making [2]. The findings from this work could be integrated into the continuous science-based support aimed at optimizing the comprehensive farm-to-fork and One Health (OH) *Campylobacter* control strategy. Follow-up cost-benefit analysis could further help driving risk-based prioritization of measures along the food chain.

4.2 Limitations of the study

Estimates of the QMRA model regarded risk of human disease per serving, and thus, eventual extrapolations in terms of national human disease incidences and their comparisons with reported public health data should be made with caution, because for that purpose, several (often uncertain) variables should be considered (e.g. proportion of the human population actually consuming broiler meat, disease underreporting factors, diagnostic tests used and their performance, etc.).

A similar caution applies if extrapolating our results to other countries. For instance, variations in MAP gas compositions and their concentrations used elsewhere may result in different per-meal risks—and different mitigation effects—than those estimated here for Denmark.

The potential impact of initial (pre-treatment) *Campylobacter* concentrations associated with different meat cuts was incorporated based on only two studies [4, 5], as these were conducted recently and under current meat-processing practices in Denmark. However, the simulated prevalence and concentration distributions were remarkably similar between ScenII and ScenIII, despite being based on reduction factors defined for neck skin and breast fillet—cuts known to exhibit substantially different contamination levels at the slaughterhouse.

Finally, it should be noted that, in this study, the two mitigation measures (or their combination) were applied simultaneously to all 1,296 flocks, in order to enable an overall comparison of their effects. In reality, however, some broiler meat produced in the country may undergo different treatments (e.g. vacuum packaging), and some cuts may also be sold directly as unpacked fresh meat (e.g. in butcheries).

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Figure 1. Odds ratios (ORs) for Campylobacter-positive flocks under the reference scenario (ScenRef) compared with mitigation scenarios (ScenI–ScenV), stratified by slaughterhouse (SA–SD) and quarter (Q1–Q4). ORs were estimated using a binomial GEE model with robust standard errors and are shown with associated 95% confidence intervals. ORs greater than 1 (dashed line) indicate that ScenRef had higher odds of carcass-flock positivity relative to the corresponding mitigation scenario. Tukey adjustments were applied to all scenario comparisons within each slaughterhouse–quarter combination.

Keywords: Campylobacter, QMRA, Risk mitigation, MAP, Slurry-ice.

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