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▶ **To cite this version:**

Francis Lauriau, Maarten Nauta, Nabila Haddad, Sofia Strubbia, Jean-Michel Cappelier, et al.. Harmonizing Campylobacter risk assessments across European countries – can the pooled process hygiene criteria data be used in the Danish risk assessment model?. Microbial Risk Analysis, 2024, 27-28, pp.100325. 10.1016/j.mran.2024.100325 . hal-04777973

HAL Id: hal-04777973
https://hal.inrae.fr/hal-04777973v1

Submitted on 12 Nov 2024

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Harmonizing *Campylobacter* risk assessments across European countries – can the pooled process hygiene criteria data be used in the Danish risk assessment model?

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Highlights

- French *Campylobacter* data 2020-21 on testing broilers neck skin (NS) pools was used
- Human risk of disease was estimated by a Danish model developed for leg skins (LS)
- The 2021 risk relative to 2020 (RR) was 1.22 meaning an increase of risk of ≈ 22%
- A harmonized risk assessment could be carried out using pooled NS data
- Limitations for harmonizing risk assessment across countries were addressed

Abstract

This study investigated the possibility of harmonizing quantitative microbiological risk assessment (QMRA) for *Campylobacter spp.* across European (EU) countries.

French *Campylobacter* data (2020-2021) from neck skin (NS) pools, sampled at slaughterhouses under the European surveillance component for Food Business Operators (FBOs), were adapted to inform a QMRA model that, among others, has been used within the Danish Action Plan against *Campylobacter*, on the basis of single leg skins (LS) data.

Datasets included culture results (in colony forming unit per gram, CFU/g) from 1,284 broiler flocks slaughtered at 13 slaughterhouses representing broiler production in western France. Five pools (of 2-4 NS samples each) per flock were tested. One pool per tested flock was randomly chosen for the analysis. After conducting descriptive statistics (on flock prevalence and meat contaminations across months and years), three contamination transformation factors (CTFs) were estimated to translate NS pools contaminations into single LS contamination, based on data from French and Danish studies. A reference simulation scenario (ScenRef) was set with CTF = 3.2 (i.e. NS pool concentration divided by 3.2); while other 13 scenarios represented an alternative scenario analysis to investigate the impact of: the CTF value (ScenMin with CTF = 2 and ScenMax with CTF = 10), censored test results (ScenUncens) and random choice of pool per flock (ScenSampling-1 to 10), on the risk estimates. The average monthly/annual risk of human disease per poultry meal and the monthly/annual relative risk (RR) of 2021 compared to 2020, were estimated.

In ScenRef, the annual RR was 1.22, suggesting an increase of risk of ≈ 22% in 2021 compared to 2020. The impact of CTFs, censored data and randomized pool sampling per flock, on the annual and (most) monthly RRs, appeared limited.

This study gives an overview of the strengths and limitations to be considered for adapting the French FBO data into the Danish model and to harmonize risk assessments across EU countries, accordingly. To reduce uncertainty in risk estimates, it could be considered increasing representativeness of NS tested flock populations and/or using LS rather than NS samples; because LS samples are more representative of actually retailed meat contaminations. If NS pools are

maintained, the relationships between concentrations on NS pools and those on consumed meat requires further investigation.

Key words: *Campylobacter*; *Neck skin pools; Surveillance data; Harmonization; Risk assessment; Contamination transformation factor*

1. Introduction

Campylobacter spp. is the most commonly reported cause of foodborne gastrointestinal infection in humans in Europe (EU). In 2022, 137,107 human cases were reported, corresponding to a notification rate of 43.1 per 100,000 inhabitants (EFSA and ECDC, 2023). In France, the notification has been rising steadily since 2017 and was at its highest in 2022 (67.0 cases per 100,000 inhabitants) (EFSA and ECDC, 2019, 2023). As the number of confirmed cases of campylobacteriosis constitutes only a portion of the cases that actually occurred (due to underreporting and underdiagnoses) (Havelaar et al., 2013; Monteiro Pires et al., 2020), the average annual number of symptomatic cases in France was estimated at ≈ 493,000, of which ≈ 392,000 are thought to be foodborne over the period 2008-2013. For those estimates, underreporting and underdiagnoses factors were applied on national reference laboratory data (Van Cauteren et al., 2017). Moreover, EFSA estimated that broiler meat may account for 20–30% of campylobacteriosis human cases in the EU, while 50–80% of these may be attributed to the chicken reservoir as a whole (EFSA BIOHAZ Panel, 2010). Campylobacteriosis is mainly characterised by gastrointestinal symptoms, but it can also cause a serious post infectious neuro-paralytic complication: Guillain-Barré syndrome (Allos, 1997).

The public health importance of *Campylobacter* contamination and survival have been investigated throughout the poultry sectors (from farm to fork) using Quantitative Microbial Risk Assessment (QMRA) models, which include a model of the consumer phase (Chapman et al., 2016). These models can be used to inform evidence-based risk mitigation actions along the food chain. In Denmark, a QMRA model developed by Nauta *et al.* (Nauta et al., 2008; Nauta et al., 2012) has been used since 2014, to assess the risk of campylobacteriosis due to *Campylobacter* contaminated fresh broiler meat. In the Danish Action Plan against *Campylobacter*, the results of culture testing single leg skin (LS) samples, are fed into this model, to assess the mean annual risk per serving (i.e. per meal) and its changes in relation to a reference year (2013) (Foddai et al., 2022a, 2023; Foddai et al., 2022b).

In 2017, a process hygiene criterion (PHC) for *Campylobacter* in broiler meat, to be complied with by Food Business Operators (FBOs), was introduced at EU level. The criterion is defined by Regulation (EU) 2017/1495 (Anonymous, 2017a) and sets the microbiological limit in colony

forming units per gram (CFU/g) for pooled neck skin (NS) samples taken from broiler flocks at slaughterhouses. Five pooled NS samples are analysed per sampling session per week.

The PHC aims to inform risk mitigation actions at the single slaughterhouses rather than at national level. Still, FBOs need to inform the competent authorities about non-conformities upon request (Anonymous, 2017b), and also to report the annual number of pooled NS samples with a concentration larger than 1,000 CFU/g (Anonymous, 2019).

If the NS surveillance data obtained from individual FBOs under Regulation (EU) 2017/1495, could be used as input in a QMRA model, there would be an important international added value, e.g. for harmonizing national quantitative risk assessments across EU countries. In fact, during recent years, there has been an increased demand for standardizing procedures of inter-sectorial data integrations, data analysis and risk assessments within the EU (Colangeli et al., 2023; One Health EJP, 2018, 2023).

The objective of this study was to give a proof of concept (with strengths and limitations to consider) for potential harmonization of quantitative risk assessment for *Campylobacter spp.* across EU countries, by using as example the Danish QMRA model (Nauta et al., 2008; Nauta et al., 2012), informed by the French NS surveillance data.

2. Materials and methods

The QMRA model used to assess the risk per serving and its relative changes across surveillance periods (months and years) requires concentrations that are representative for single carcasses, not from pooled broiler samples. Therefore, in the risk assessments done for the Danish Action Plan (Foddai et al., 2022a, 2023), single LS samples are used. Those are more representative for the carcass contaminations to which the consumer is actually exposed (usually, NS are not sold for consumption, although sampling does not represent any meat loss to the FBO). As a consequence, the *Campylobacter* concentration quantified on the French NS pooled samples had to be transformed into concentrations for single LS samples, before being fed into the model. Data handling and analyses were carried out using the package *"base"* in R version 4.3.1 and RStudio software version 2023.6.2.561 (Posit team, 2023; R Core Team, 2023), whereas the risk assessment was carried out using the Danish QMRA model (Nauta et al., 2008; Nauta et al., 2012).

2.1 The original data on pooled NS samples

Data 2020-2021 from thirteen slaughterhouses were used for the study. These are located in western France and were estimated to represent approximately 48% of the French tonnage of broilers slaughtered in 2021 (Agreste, 2022). The metadata included: the date of slaughter (i.e. sampling), the identifier of the broiler flock, the analysed food matrix (i.e. NS pools) and the *Campylobacter* concentration according to the culture standard EN ISO 10272-2 (ISO 10272-2, 2017). A flock was defined as the group of broilers reared in the same house unit of the same farm (conventional or organic), and slaughtered on the same day.

The data was collected in Excel files (version Excel 2021) in two stages. Firstly, testing results were collected directly from five slaughterhouses´ quality department teams. Secondly, for the other eight slaughterhouses, the surveillance results were extracted from the Donavol platform of the French federation of poultry industries (in French: Fédération des Industries Avicoles (FIA)) (FIA, 2020). This federation is responsible for collecting and transmitting to the Ministry of Agriculture the self-checking data required by the regulations in poultry slaughterhouses (Anonymous, 2017a, 2017b, 2019).

According to Regulation (EU) 2017/1495 (Anonymous, 2017a), five pools of NS samples (a pool is made of up to four carcasses from the same flock of origin) must be analysed at each slaughterhouse, each week of the year. In practice, the five NS pools come from the same flock. There may be three main types of pooled NS samples, according to regulations and analyses to be performed (Anonymous, 2017a, 2021): a) Pools of two samples when used solely for *Campylobacter* testing; b) pools of three samples when used for *Salmonella* and *Campylobacter* testing in the same laboratory; and c) pools of four samples when used for *Salmonella* and *Campylobacter* testing in different laboratories. In the data used for this study, each pool was obtained from 2-4 NS single samples (i.e. all options: a, b, and c were applicable).

In total, the data consisted of 6,574 NS pools collected from 1,284 flocks, over the two years: from 620 flocks in 2020 and from 664 flocks in 2021. For 14 of the flocks, one to four NS pools out of five were not available due to logistical or sample analysis problems. For other 36 flocks, 10 NS pools were tested.

2.2 Standardization of concentration ranges across slaughterhouses

For the eight slaughterhouses which did not provide the data directly (Section 2.1), the *Campylobacter* counts <100 and >10,000 CFU/g were censored in the Donavol database, because the FBOs are not required entering CFU/g below or beyond these values (FIA, 2020). In a context of adaptation of individual FBO data into a national level risk assessment, for France, the Donavol database would represent the main national data source and format. Therefore, all the datasets from the thirteen FBOs were standardized similarly, to represent at national level, the overall data analysis (Section 2.3) and the simulation reference scenario (Section 2.4-2.5). Accordingly, the NS pools recorded as "<100" CFU/g were set as = 0 (i.e. considered negative) and the pools recorded as ">10,000" CFU/g were set equal to 10,000 CFU/g. Concentrations in between these two limits were kept as originally recorded and classified positive[1].

2.3 Descriptive statistics and statistical data analysis

From the standardized data, for each tested flock, one of the five NS pools was randomly selected using the function *"sample"* in the package *"base"* in R (R Core Team, 2023), to simulate the random selection of the leg sampled per flock at the abattoir in the Danish Action Plan. Thus, the overall number of pools selected for the descriptive statistics was 620 for 2020 and 664 for 2021.

The procedure and the interface tool from Foddai et al. 2024 (under review), were used to visualize and interpret outputs of data analysis, together with their related risk estimates (see Section 2.5). The descriptive statistics included: the number of tested and positive flocks, with their corresponding apparent prevalence (AP), i.e., the prevalence not corrected for the diagnostic test´s error (Rogan and Gladen, 1978; Moreno-Torres et al., 2016; Foddai et al., 2022b). Moreover, the distributions of concentrations across the positive NS pools were $\log_{10}$ transformed and compared between surveillance months and years, through box plots produced in R.

A multiple linear regression model was also set for statistical analysis (significance level at P-value < 0.05) to compare $\log_{10}$ *Campylobacter* concentrations across the two surveillance years and the thirteen slaughterhouses, and to assess whether there was any interaction between abattoir

[1] N.B. The impact of this data standardization procedure is addressed through the alternative scenario analysis, in Section 2.6.

and year. For that purpose, the "*lm*" function of the R statistical package "*stats*" was used to set a linear regression model (Wilkinson and Rogers, 1973; Chambers and Hastie, 1992) as:

$$log_{10}\ concentration = ß_0 + ß_1\ year + ß_2\ slaughterhouse + ß_3\ year * slaughterhouse + \varepsilon_i.$$

The term "*year * slaughterhouse"* represented the interaction between these two variables. The results of the model were observed using the function *"summary"* from the package R *"base"*. However, the significance of each overall variable was assessed by analysis of variance (ANOVA) using the function *"anova"* from the package *"stats"*. Moreover, the suggested model according to the AIC criterion was also checked using the *"drop1"* function from the package *"stats"* (Chambers and Hastie, 1992). Both variables were categorical. For the variable *"year"* the 2020 was set as reference category, while abattoir No.4 (Ab_04) was set as reference for the variable *"slaughterhouse"*. These were the categories with the lowest log_{10} concentration across the two years.

2.4 Transforming concentration from neck skin pools into (simulated) single leg skin contaminations

In France and Denmark, two publications examined the different *Campylobacter* concentrations between paired pooled NS samples vs. single LS samples (Duqué et al., 2018; Ellis-Iversen et al., 2020). In both cases, the concentrations of *Campylobacter spp.* found on the single LS was lower than these found on the pooled NS. The data from both studies were used to estimate the contamination transformation factors (CTFs), which enabled the approximation of single LS concentration from the NS pools concentration.

2.5 The quantitative risk assessment model and the reference risk assessment scenario

The risk assessment model (Nauta et al., 2012), as applied in Denmark (Foddai et al., 2022a, 2023), relates the apparent prevalence (AP) of carcass positive flocks and their *Campylobacter* concentrations detected on single LS samples collected at slaughterhouses, with the risk of human campylobacteriosis per broiler meat serving (i.e. per meal). Accordingly, the model describes:

i) The difference between concentrations on single LS samples and broiler meat (after skin removal) (Nauta et al., 2012),

ii) The effect of preparing a meal with broiler meat, on the bacterial transfer and survival, so that the ingested dose can be estimated, as described in an observational study (Nauta et al., 2008; Nauta and Christensen, 2011), and

iii) A widely used dose-response model for *Campylobacter* (Teunis and Havelaar, 2000).

The original version of the risk assessment model is a stochastic model that captures the variability between servings (Nauta et al., 2012). Here we use the association between the input and the output of this model, which provides a deterministic numerical relationship between the LS concentrations and the mean probability of illness per serving (Figure 1), as found after 91×50,000 iterations in a Monte Carlo simulation of the stochastic version (Nauta et al., 2012). This deterministic relationship is also used in the Danish Action Plan and has been previously applied in different studies, as a generic risk assessment model for *Campylobacter* based on broiler skin data (EFSA BIOHAZ Panel, 2011; Nauta and Christensen, 2011; Nauta et al., 2012; EFSA BIOHAZ Panel et al., 2020). We refer to Nauta et al. (2012) for a more detailed description.

For our purpose and within the Danish Action Plan, the model provides estimates of the average monthly risk per serving based on monthly collected skin samples, as well as the mean annual risk, which is equal to the mean of the 12 monthly risk estimates of the same year. Next, the monthly and annual relative changes in risk (i.e. the relative risk (RR) estimates) can be calculated, by comparing two years. In this study, 2020 was used as year of reference (or baseline) and the monthly/annual RRs were estimated for 2021; by dividing the monthly/annual risk estimates from 2021 by these of 2020.

An RR <1 or >1 suggested a decrease or an increase (respectively) of the monthly/annual risk of 2021, compared to that of 2020, whereas RR = 1 suggested no change in risk across the months and years.

For the simulation reference scenario (ScenRef), the CTF was derived from Ellis-Iversen et al., (2020), where the mean concentrations were estimated = 886 CFU/g for the pools of three NS samples and = 276 CFU/g for the LS single samples, so CTF = 886/276 = 3.2. Accordingly, in the ScenRef, the estimates for the single LS concentrations were obtained by dividing the concentrations observed in the (randomly) selected NS pools by 3.2. Accordingly, by using the

CTF values > 1 it was reflected the fact that: a) the NSs are usually more contaminated than LSs, due to the carcass position (upside down) in the slaughter line, which makes the former more likely to become contaminated (e.g. due to viscera ruptures); and b) that the mean concentration in a pooled sample is expected to be larger than that in a single sample, when the distribution of concentrations is lognormal. Next, within the model, the LS simulated concentrations were $\log_{10}$ transformed.

2.6 Alternative scenario analysis

An alternative scenario analysis was conducted, to examine the sensitivity of RR estimates (the main output of interest) due to three key factors: a) CTFs (i.e. on the simulated single LS contaminations); b) censored data; c) randomized pool sampling (i.e. the random selection of one NS pool from five per flock). Therefore, thirteen alternative risk assessment scenarios were compared to the ScenRef (Section 2.5):

I) two scenarios were carried out using minimum CTF = 2 (ScenMin) and maximum CTF = 10 (ScenMax), based on the data from the French and the Danish studies (Duqué et al., 2018; Ellis-Iversen et al., 2020);

II) one scenario (ScenUncens) replaced the originally NS censored results recorded as "<100" CFU/g and ">10,000" CFU/g in the standardized data (which was used also from the ScenRef), with values sampled from two distinct PERT distributions, which were set in R using the function *"rpert"* in the package *"mc2d"* (Pouillot and Delignette-Muller, 2010). These distributions were defined using the data from the five slaughterhouses quality departments, which had broader CFU/g ranges available (as described in Section 2.1). This scenario allowed assessing the impact of the censored data ("<100" and ">10,000") and of the data standardization procedure described in Section 2.2, on the risk estimates. Therefore, here a broader range of concentration values were included, compared to all other scenarios: specifically, also contaminations below 100 CFU/g and above 10,000 CFU/g. Hence, for all 13 slaughterhouses, contaminations between 100 and 10,000 CFU/g were used. Next, for the five slaughterhouses having values below 100 and above 10,000, all the original contaminations (from 10 to 150,000) were used (i.e. these given by the companies and before the data standardization made in Section 2.2), and censored data "<10", ">15,000" or ">150,000" were replaced by 0, 15,000 and 150,000 respectively. For the remaining eight

slaughterhouses for which data was extracted from the Donavol database, the censored values reported as "<100" and ">10,000" were replaced by simulated values according to information from the other five companies and the mentioned PERT distributions, to decide which value to use into the censored data cells. Accordingly, a PERT distribution was based on contamination data below 100 CFU/g (i.e. PertLow: min = 0; median = 10; max = 99) and another on the data exceeding 10,000 CFU/g (i.e. PertHigh: min = 10,001; median = 25,000; max = 150,000). Thereafter, all concentrations (simulated and not) were divided by CTF = 3.2 (as in the ScenRef) and flocks with simulated LS results below 10 CFU/g were considered as negative and set at "0" into the model, as is currently (mid 2024) the case within the Danish Action Plan.

III) ten scenarios, each based on a different random sampling of NS pool per flock, were created but still using CTF = 3.2 (ScenSampling-1 to ScenSampling-10).

Accordingly, scenarios ScenMin, ScenMax and ScenSampling (1 to10) investigated the impact of between and within-flock contaminations variability on the risk estimates, while ScenUncens investigated the impact of censored data.

3. Results

Sections 3.1 and 3.2 present the results from the descriptive statistics and from the data analysis (i.e. the AP estimates and the concentration distributions), while Section 3.3 shows the results of the risk assessment (i.e. the risk and the RR estimates) under the reference scenario. Finally, Section 3.4 presents the outputs of the alternative scenario analysis.

3.1 Apparent prevalence (AP) of positive flocks

Across the two investigated years, according to the culture testing results from the randomly selected NS pools of the standardized data (Section 2.2) used in the ScenRef (Section 2.4-2.5), the overall prevalence of positive flocks (i.e., of sampled pools ≥ 100 CFU/g) was 69.5% (892/1,284). The annual prevalence was 68.7% (426/620) in 2020 and 70.2% (466/664) in 2021.

Across the two years, the overall monthly median number of tested flocks was 52 (min = 45 in February and max = 59 in December) and 55 (min = 49 in October; max = 62 in March), respectively (Figure, 2A).

The overall monthly median number of positive flocks was = 36 in 2020 (min = 22 in February; max = 45 in June) and 40 in 2021 (min = 25 in January; max = 52 in August) (Figure, 2B).

The overall monthly median AP was 69.4% (min = 48.9% in February; max = 84.9% in June) and 72.5% (min = 47.2% in January; max = 89.7% in August), during 2020 and 2021, respectively. In both years, the monthly AP showed seasonality and increased during summer months, compared to the rest of the year (Figure, 2C).

3.2 Concentration distributions on standardized NS pools according to surveillance period

Considering the positive flocks shown in Figure 2B, across the two investigated years, the overall mean *Campylobacter´*s concentration was 3.03 $\log_{10}$ CFU/g (2.5th percentile = 2.00; 1st quartile = 2.46; 3rd quartile = 3.67; 97.5th percentile = 4.00). During 2020 and 2021, the overall mean concentrations were 2.96 $\log_{10}$ CFU/g (2.00; 2.42; 3.54; 4.00) and 3.10 $\log_{10}$ CFU/g (2.00; 2.52; 3.75; 4.00); respectively.

In the multiple linear regression model, the interaction between the two variables "*year*" and "*slaughterhouse*" appeared significant only for the two combinations *"2021 * Ab_01"* and *"2021 * Ab_08"* ($p < 0.05$); whereas the overall interaction did not appear significant by ANOVA ($p = 0.1799$) and was suggested for removal from the model based on the AIC criterion. Without considering this interaction, the difference in mean concentrations between the two years appeared significant by ANOVA ($p < 0.001$), as did the difference between abattoirs ($p < 0.001$).

A visual comparison of the concentration distributions, across monthly surveillance periods, is shown in the boxplots from Figure 3. It can be noted that no particular seasonal patterns were observed in any of the two investigated years. The months with bigger difference in mean $\log_{10}$ concentrations across years, were June (higher for 2021) and December (higher for 2020). Furthermore, the mean standard deviation of concentration between positive NS pools of the same flock was 0.31 $\log_{10}$ CFU/g (min = 0.00; 2.5th percentile = 0.00; 1st quartile = 0.15; 3rd quartile = 0.44; 97.5th percentile = 0.76; max = 1.10), indicating the variability across NS positive pools contamination levels within flocks.

3.3 Risk and relative risk (RR) estimates according to reference scenario

Under the reference simulation scenario (ScenRef with CTF = 3.2 and censored data <100 and >10,000 CFU/g), during 2020, the monthly risk (i.e., the average probability of campylobacteriosis per broiler meat meal) ranged from 0.35% in January to 0.98% in May (Figure 4A, middle green line). The overall mean annual risk (across the 12 months) was 0.67%. In 2021, the monthly risk ranged from 0.44% in January and 1.16% in June (Figure 4A, middle yellow bar), while the overall annual average risk was 0.82%.

During 2021, the RRs were < 1 in four months (March, May, November and December) and >1 in the remaining eight months of the year (Figure 4B, middle yellow bars). Between those (in hierarchical order) June, October and April showed the highest RRs. During the year, the monthly RR ranged from 0.75 in December (i.e. showing a reduction of 25% in risk compared to December 2020), and 1.96 in June (i.e. almost doubled risk).

The overall annual RR obtained by the ratio of the two annual risk estimates was 0.82% / 0.67% = 1.22; thus indicating for 2021, an overall increase of risk per serving of ≈ 22% compared to 2020 (Figure 4C, middle yellow bar).

3.4 Outputs of alternative scenario analysis

3.4.1 Impact of concentration transformation factors (CTFs)

The alternative scenarios ScenMin (CTF = 2) and ScenMax (CTF = 10) showed the impact of the assumptions made for the CTF on the risk estimates (i.e. when transforming the *Campylobacter spp.* contaminations observed in the NS pools, into the simulated single LS contaminations) (Figure 4A). For example, in May 2020, which was the month with the highest risk during that year, the risk changed from 0.98% in the ScenRef to 1.23% in the ScenMin and 0.52% in the ScenMax (Figure 4A, green lines). For the three scenarios, the average annual risk (across the 12 months) was 0.67%, 0.86% and 0.35%; respectively. Instead, for May 2021, estimates changed from 0.76% (ScenRef) to 0.97% (ScenMin) and 0.40% (ScenMax) (Figure 4A, yellow bars). In that year, the average annual risk was 0.82%, 1.04% and 0.42%; respectively.

The effect of the assumed CTFs, on each monthly RR of 2021, is shown in Figure 4B, by comparing the numbers reported over the three yellow bars of the same month. The biggest

variation of RRs occurred in June, when changing from the ScenRef (RR = 1.96) to the ScenMax (2.18).

Regarding the annual RR (the main output of interest), it changed from 1.22 in the ScenRef to 1.21 and 1.26 in the ScenMin and ScenMax scenarios (Figure 4C).

3.4.2 Impact of censored data

The scenario ScenUncens demonstrated the impact of replacing the censored concentrations (i.e. concentrations recorded as "<100" CFU/g and ">10,000" CFU/g), with values sampled from the PERT distributions (which were set as described in Section 2.6).

This is the only scenario that, next to the concentration distributions, affected also the prevalence of simulated LS positive flocks, because concentrations between 10 and 100 CFU/g were also considered positive. For instance, in ScenRef, the flocks with censored values "<100" were translated into LS contaminations = 0 (i.e. <10 CFU/g and LS negative). In contrast, in the ScenUncens, the same censored flock could get a value >0 (e.g. 90 CFU/g from the PERT distribution), which could give a LS simulated concentration >10 CFU/g (e.g. 90/3.2 = 28 CFU/g, i.e. LS positive).

Thus, the number of positive flocks per month was higher in the ScenUncens for 2020 (median = 41) and 2021 (median = 43) compared to the ScenRef (median = 36 in 2020, 40 in 2021). Consequently, the monthly and the annual LS-AP were also consistently higher in the ScenUncens – varying between 62.2% and 90.6% in 2020 and between 50.9% and 93.1% in 2021 – than in the ScenRef (where it ranged from 48.9% to 84.9% and from 47.2% to 89.7%, respectively).

The simulated LS mean concentrations also increased for both years, when passing from the ScenRef (2.74 $\log_{10}$ CFU/g for 2020 and 2.86 $\log_{10}$ CFU/g for 2021) to the ScenUncens (3.12 $\log_{10}$ CFU/g in 2020; 3.22 $\log_{10}$ CFU/g in 2021).

Accordingly, the monthly risk was always higher in the ScenUncens than in the ScenRef in both 2020 (compare green lines in Figure 5A) and 2021 (compare yellow bars in Figure 5A).

The monthly RRs were higher in the ScenUncens than in ScenRef for February, March, April, June, July, and October, but lower for the remaining months. The largest increase in monthly RRs

was observed for June (RR = 1.96 in the ScenRef and 2.18 in the ScenUncens), while the largest decrease was seen for September (1.33 and 1.12) (Figure 5B). The annual RR changed from 1.22 to 1.20 when comparing the ScenRef vs. the ScenUncens.

3.4.3 Impact of randomized sampling of one NS pool per flock.

Ten scenarios (ScenSampling-1 to 10) were generated based on repeated random selections of one NS pool per flock out of the five tested, and simulated into single LS contaminations with CTF = 3.2. Thus, these ten alternative scenarios illustrated the variability in RR, which was due to the selection of a single result out of five available per flock, to simulate LS contamination. The largest monthly RR difference between ScenRef and the ten ScenSampling scenarios were observed for March (largest increase from ScenRef RR = 0.94 to ScenSampling-3 RR = 1.40) and for June (largest decrease from ScenRef RR = 1.96 to ScenSampling-7 RR = 1.52) (Figure 6).

Finally, the annual median RR for 2021 was 1.23, varying from 1.18 to 1.28 across the ten scenarios, and representing a maximum variation of +/- 5% compared to the ScenRef (1.22).

4. Discussion

We studied the use of the NS surveillance data collected at slaughterhouses under Regulation (EU) 2017/1495, for an harmonized quantitative risk assessment approach for *Campylobacter spp.* across different EU countries, i.e. by using French surveillance data in a Danish procedure for risk assessment (Nauta et al., 2008; Nauta et al., 2012; Foddai et al., 2022a, 2023; Foddai et al., 2022b; Foddai et al., 2024 under review).

The main advantage of having harmonized data analysis and related risk assessments at national level, would be that the risk estimates become more comparable between countries, alongside their related flock prevalence and meat concentrations. Such a harmonization can be possible when countries use the same national farm/flock/slaughterhouse populations' coverage, type of samples and diagnostic test. A good candidate for such a harmonization would be Regulation (EU) 2017/1495. The purpose of the sampling described on the EU regulation is however different, which leads to some complications when applying the collected surveillance data (i.e. NS pooled samples) for the mentioned risk assessment.

Therefore, in the Sections below, are discussed the main results of the study (Section 4.1) with the related sources of uncertainty (Section 4.2) and limitations to be considered for harmonizing the estimation of flock prevalence, carcass contaminations and risk of human disease, at national level. Finally, suggestions for potential solutions to address the mentioned limitations are provided in Section 4.3.

4.1 Interpretation of outputs according to reference simulation scenario

Currently, for France, the reference simulation scenario (ScenRef), would be the most realistic, if the Donavol data format was kept unchanged and is used for all flocks tested in the country (i.e. if culture results <100 and >10,000 were kept as censored). The impact of such data format, was investigated in the alternative scenario analysis (see next section).

According to the annual RR of the ScenRef, the average annual risk per serving increased notably (by almost one fourth) for 2021 compared to 2020 (Figure 4C). This relative increase of annual risk, partly aligns with the increased number of confirmed cases of campylobacteriosis in France in 2021 (8875) compared to 2020 (7920) (EFSA and ECDC, 2023).

Nevertheless, the increase of risk was not homogeneous during the 12 months of 2021 (Figures 4A-B), neither did it show a particular seasonality. Actually, in one third of the surveillance months (i.e. in 4 out of 12), the RR was <1. Therefore, the data and the model used in this study, allowed identifying the surveillance periods during which the most important changes (and especially) the highest relative increases of risk, occurred during the year of interest (2021).

For instance, looking at April, June, and October it can be noted that those months showed the highest RRs (Figure 4B). Accordingly, a more detailed investigation can be carried out retrospectively, to understand if the mentioned relative increases of risk, were caused by an increased prevalence (namely the AP) and/or by increased *Campylobacter* concentrations (namely the monthly CFU/g distributions), in line with (Foddai et al., 2024, under review). In fact, the risk estimates used to calculate the RRs, are affected by the combination of both the AP and the *Campylobacter* concentration distributions fed into the QMRA model (Nauta et al., 2008; Nauta et al., 2012; Foddai et al., 2022a, 2023, 2024 under review). From previous risk assessments for *Campylobacter*, it is known that the variance in risk is most often affected by the variance in

prevalence rather than by the variance in concentrations (FAO, 2001; Christensen et al., 2001; Nauta and Havelaar, 2008; Nauta et al., 2009; Foddai et al., 2023). Still, relatively high meat concentrations can also cause high risk values during some surveillance periods (even when the AP is relatively low).

In this study, the overall annual AP was higher during 2021 than in 2020, and during both years, the monthly AP showed a seasonal pattern (Figure 2C), similarly to other countries like Denmark (Kuhn et al., 2020; Foddai et al., 2022b), where the flock prevalence is known to increase during summer months. From the descriptive statistics, it can be seen that the monthly flock sample sizes were similar between the months and years (Figure, 2A), and thus, the observed seasonal changes of AP, represented actual epidemiological patterns in the monthly occurrence of *Campylobacter* positive flocks (Figure 2B) within the population of studied French flocks.

At the same time, for the meat concentration distributions, no particular seasonality was observed within the two years (Figure 3). When looking at the three months mentioned above (those with the worst RRs), the boxplots from Figure 3, showed higher median $\log_{10}$ concentration in 2021 than in 2020. Accordingly, it could be concluded that, for April and June 2021, the RR>1 was caused mainly by the worsening of concentration distributions, rather by relevant increases in AP. Whereas for October, both parameters increased and contributed to the worsened risk per serving of 2021 compared to 2020. Consequently, for all the three months, it could be also speculated that the relative increase of risk was mainly caused by flocks which arrived at the slaughterhouse already contaminated (from the farm). In fact, usually, flocks contaminated from the pre-harvest are those contributing the most to the AP detected at slaughterhouses and have significantly higher concentrations than flocks cross-contaminated on carcasses during slaughter. In Denmark for example, cross-contaminated flocks represented approximately 8-9% of all carcasses positive flocks and had a median concentration which was relatively low compared to flocks originally contaminated from the farm (Foddai et al., 2022b).

The meat contaminations were also studied using the multiple linear regression modelling based on year and slaughterhouse set as categorical variables. The interaction between these two variables appeared significant only for a few combinations (2021 with Ab_01and 2021 with Ab_08) and the number of observations was similar for both slaughterhouses across both years (Ab_01: 33 in 2020, 35 in 2021; Ab_08: 41 in 2020, 38 in 2021). Hence, based on these data, the

mentioned few interactions apparently lack a biological explanation if we only consider the year and the slaughterhouse in the model. It is therefore not possible excluding that unaccounted factors, such as flock type (e.g. organic vs. conventional) and/or their origin (i.e. farm´s effect), might have partly affected the results of the statistical analysis. On the other hand, since the overall interaction did not appear significant; if the two individual factors (year and slaughterhouse) were considered independently, it seems that both significantly affected the NS contaminations and year 2021 showed a significantly higher mean $\log_{10}$ concentration than 2020.

All this information obtained from the combination of data analysis and risk assessment, when interpreted systematically and retrospectively, across surveillance periods (e.g. year by year and/or month by month) allows tracing the flocks and their farms/house units of origin (through the ID metadata) which contribute the most to the AP, to the concentrations on the meat, and consequently to the risk of disease in a country (Foddai et al., 2024 under review). Hence, if needed, the risk assessment can also be carried out at individual slaughterhouse level (using the respective surveillance data), so that the FBO contributing the most to the overall national risk (and the farms delivering them the slaughtered flocks) can be traced across surveillance periods and be prioritized for additional surveillance and/or risk-based control actions along the food chain, as previously investigated for Denmark (Foddai et al., 2022a, Foddai et al., 2023, Foddai et al., 2024 under review).

4.2 Information from alternative scenario analysis

The impact of the CTFs appeared limited, especially on the annual RRs (Figure 4C). The biggest variations in monthly RRs, across simulation scenarios (namely ScenRef vs. ScenMin and ScenMax); were observed in June when (as mentioned above) the largest difference in the $\log_{10}$ mean concentration was observed between the two years (Figure 3). Therefore, assumptions on CTF can have higher impact on the RR, when differences in concentrations are larger across the considered periods.

The scenario ScenUncens, showed that the initial data standardization procedure used in the ScenRef (i.e. setting contaminations = 0 and = 10,000 CFU/g for cells having original censored records as: "<100" and ">10,000" CFU/g, respectively) resulted in an underestimation of the risk,

for both 2020 and 2021 (Section 3.4.2). These differences between risk estimates of the same year, by changing from ScenRef to ScenUncens, were due to the differences in the LS simulated flock prevalence and concentrations. In fact, the considered concentration distributions had a larger range (from 10 to 150,000 CFU/g) and a larger upper limit (>10,000) in the ScenUncens than in the ScenRef. Consequently, the overall concentrations and the simulated LS-AP were higher in ScenUncens than in ScenRef, due to the higher frequency of flocks, which were simulated as carcass positive in LSs (i.e. also with values between 10-100 CFU/g and >10,000). Nevertheless, when combining the effect of the simulated LS-AP and meat concentrations, on the risk of both years, the impact of the censored data on the annual RR appeared marginal (the RR reduced of 2% on the ScenUncens compared to the ScenRef, Section 3.4.2), while it was more evident on the monthly RRs (Figure 5B). For instance, for June, the RR of the ScenUncens was remarkably higher than that of the ScenRef (Figure 5B: RR 2.18 vs. 1.96), because in that month, the simulation of concentrations for the censored data, increased more the risk of June 2021 than that of June 2020 (used as baseline). In contrast, for September, the opposite situation occurred (Figure 5B: 1.12 vs. 1.33).

The variability of RR estimates associated with the random selection of one pool out of five per tested flock, was investigated in scenarios ScenSampling-1 to 10. The impact of this pools selection process on the annual RR appeared limited (RR +/- 5% compared to the ScenRef) (Figure 6), while the effect was more noticeable for monthly RRs due to variations in AP and *Campylobacter* counts; particularly in March, June, September and October, when there was a wider range of RRs (Figure 6). This variability across the ScenSampling scenarios, occurred because fluctuations in *Campylobacter* counts within flocks can happen, as also shown by the standard deviation distributions observed across standardized NS positive pools of the same flock (Section 3.2) and as also observed in a previous study based on skin sample data (with more than one sample taken per flock) (Allen et al., 2007). Such variations affected both the simulated LS monthly contaminations and flock prevalence, and consequently, the variability of RR estimates.

Overall, the alternative scenario analysis shows that the annual RR estimates ranged from 1.21 to 1.26 when comparing different CTFs, from 1.20 to 1.22 when considering the usage of censored data, and from 1.18 to 1.28 when considering the random selection of one pooled NS sample per flock. This implies that the major simplifications used (a fixed ratio of single LS and pooled NS

concentrations, the simulation of censored data, and the usage of a randomly selected single pooled NS sample) have little effect on the main output of the model; namely the annual RR. On the other hand, the current FBO data format and its coverage of national flock populations, can impact the uncertainty on prevalence, contaminations and monthly RR estimates. Such an impact could be mitigated by addressing the data limitations, which is suggested before applying/comparing the shown procedures of data analysis and risk assessment across EU countries and to achieve a reliable level of international harmonization and comparability of results (see next section).

4.3 Limitations and potential solutions for eventual future international applications

The *Campylobacter* surveillance data used for the study was collected by individual FBOs on a weekly basis, following EU Regulation 2017/1495. As explained in the introduction, such data is aimed to inform corrective actions at the single slaughterhouses (i.e. when the PHC is exceeded) and is not primarily collected to estimate flock prevalence, carcass concentration distributions and risk of human disease, at national level. For those purposes, flock sample sizes should be set considering test´s performance at the flock level (flock sensitivity and specificity), flock population's sizes and expected flock prevalence in the country.

For instance, in the used data, approximately 650 flocks per year were tested in NS pools collected from 13 slaughterhouses, which would correspond to approximately 52-53 flocks per FBO or per month (Figure 2A), out of several hundred slaughtered. To our knowledge, we estimated that the annual number of flocks slaughtered by the thirteen French FBOs was at least around 17,000 (based on 5 flocks a day for a 5-day slaughtering week over 52-week year). This would mean that the annual number of tested flocks used in this study represented only $\approx 650/17{,}000 = 3.8\%$. Hence, by using the sample sizes set at the FBO level, there is a limitation that the NS tested flocks could be poorly representative of the overall population of flocks processed in a country, when the monthly/annual flock prevalence needs to be estimated at national level. For that reason, in this study, we avoided comparing the current risk estimates from Denmark and France, which would be obtained with the same simulation model; but with different levels of surveillance coverage of the respective national populations of: farms, flocks and slaughterhouses.

Uncertainty on mean RR estimates, could also be due to sample sizes, as incidental high concentrations, which are expected when the distribution of concentrations is lognormal, have large effect on the risk estimates (Nauta and Havelaar, 2008). Accordingly, if the data analysis and the risk assessment proposed in this study, were to be standardized across EU countries, sample size calculations should be set appositely for the purposes of prevalence and concentration estimation as well as for risk assessment at national level (i.e. reflecting the aimed confidence and precision on the estimates). For example, if different tests are used, the impact of their performance should be considered, by comparing across years and countries, the "true prevalence" estimates rather than APs (Foddai et al., 2022b). Next, a stochastic version of the QMRA model could be developed to assess the uncertainty around the estimated mean RRs. Such a model could also allow estimating the statistical significance of changes in risk across surveillance periods. However, the deterministic version of the model (used in this study) could be also considered sufficient enough, for informing decision making within the same country (as in the Danish Action Plan), if the QMRA is not aimed to produce statistically significant RR estimates, which need to be generalized across different years (i.e. if the RR is repeatedly assessed on annual bases) and/or if RR estimates are not aimed for comparison across countries and the sample size of tested flocks /slaughterhouses remains similar across surveillance periods.

Moreover, to apply the same risk assessment procedure across countries, there is a need to report all the *Campylobacter* counts (CFU/g) from the laboratories into the national databases (i.e. avoiding censured data), so that similar cut-offs of the concentrations can be used internationally, to define a flock as positive or negative, and to standardize risk assessment procedures. This would remove the limitations highlighted when comparing the ScenRef vs. the ScenUncens. Currently, according to Regulation (EU) 2017/1495, only enumeration is carried out, resulting in some censored data (< 100 CFU/g and > 10,000 CFU/g), and no detection by enrichment analysis needs to be performed simultaneously on the same samples. It was therefore impossible to determine whether *Campylobacter* was absent from a sample, or whether its concentration was below 100 CFU/g.

It should also be kept in mind that the NS pools are not fully representative of the carcass contaminations, because necks are not usually consumed, and most often, a single meal is prepared from a single carcass. NS pools are usually more contaminated and allow having higher testing

sensitivity than the single LSs samples (Ellis-Iversen et al., 2020), but the latter would be more representative of the actual *Campylobacter* concentrations present on the chilled carcasses. Using a single CTF to describe the relation between concentrations in pooled NS samples and single LS samples is a simplification, as the data and theoretical considerations show that the relation is more complex (Evers and Nauta, 2001; Bahrndorff et al., 2015; Ellis-Iversen et al., 2020). We could not check the impact of having different number of necks included in the pool (as described in Section 2.1), because the used surveillance data, only represented testing results at pool level. Nevertheless the use of the CTFs, allowed updating the AP, the CFU/g distributions and the risk estimates accordingly and simultaneously, when adapting the NS data for a model, which has been previously used with LS data. However, despite economic considerations, there would be no need to estimate the CTFs if LS surveillance data could be used (as is currently the case in Denmark), and thus removing the limitations highlighted when translating NS pools contaminations into retailed-skin-carcass contaminations.

Regarding, the random selection of NS pools, it varied for some flocks due to the number of pools, which differed from the standard five, for 50 out of 1,284 flocks. This small proportion (<4%) is unlikely to significantly bias the conclusions of this study.

Furthermore, it needs to be mentioned that extrapolations of the risk estimates (e.g. from Fig. 4A) in comparison with number of reported human cases data, should be made with high caution for several reasons. For instance, a relevant level of underdiagnoses and underreporting occurs for the estimated human incidence of disease (Havelaar et al., 2013; Monteiro Pires et al., 2020). Moreover, in the QMRA model used for the study, there is a margin of error due to the assumptions and/or the lack of data needed to represent some of the simulated variables. The model includes a consumer phase section that evaluates bacterial transfer and survival when preparing a meal with broiler meat, based on a Dutch study (Nauta et al., 2008). This section was found to perform similarly (but not identically) to other consumer phase models (Nauta and Christensen, 2011); and is not necessarily representative of all European poultry meat preparations. Additionally, as explained in EFSA (2020), the majority of *Campylobacter* QMRA studies, including the previous EFSA opinion (EFSA BIOHAZ Panel, 2011), have applied the "classic" dose-response model published by Teunis and Havelaar (2000), based on a human challenge study (Black et al., 1988) in which the *Campylobacter´*s strain A3249 was used. Such a strain is not necessarily a

representative strain. New dose-response models have been proposed (Teunis et al., 2018), since the development of the model utilized in this study, and thus, the uncertainty about these parts of the risk assessment should be kept into consideration (EFSA BIOHAZ Panel et al., 2020; Nauta et al., 2022), for eventual future standardizations across EU countries, of risk assessments based on the Danish QMRA model.

Moreover, from a general point of view, it should be reminded that QMRA models tend to overestimate the risk (Havelaar et al., 2008), compared to results from other kind of epidemiological studies based on testing data; for example if the QMRA model does not account for uncertainty on consumer´s acquired immunity and/or it does not include other epidemiological factors affecting the dose-response patterns.

Finally, it must be noted that, due to the different sources of uncertainty on the risk estimates, in this study (as in the Danish Action Plan), the main research focus was addressed to the RR rather than based on the risk estimate. In the RR, uncertainties due to the consume-phase and dose-response sections, are largely cancelled out. Hence, from a managerial point of view, it can be assumed that the estimated RR and (i.e.) most change in risk across surveillance periods (years/months), will be caused by actual epidemiological changes in prevalence and/or contamination at the farm and slaughterhouse level; where regulatory control actions can be taken to reduce risk of human illness. Possible changes in (the dynamics of) transfer and survival of *Campylobacter* in the post-slaughter food chain stages were disregarded, because systematic surveillance data on such changes are lacking, and they are not easily enforced by regulations. Still, such a kind of surveillance information collected between the slaughterhouse and the food-consumer levels, could improve the risk assessment process aimed to inform inter-sectorial decision making along the food chain.

5. Conclusion

This study provides a proof of concept for the potential harmonization of data analysis and related risk assessment for *Campylobacter spp.*, across European countries using the FBO data. We showed how pooled NS data collected following Regulation (EU) 2017/1495 in France were applied in a QMRA model, which is used with single LS data in Denmark, following a procedure

633 that allows direct practical risk management support for decision makers. The impact of the
634 assumed CTFs, the censored data and the selection of a single NS pool per flock appeared limited
635 on the annual RR. Advices on potential strengths, limitations and needs for using NS data collected
636 by individual FBOs, were provided. The study can pave the road for applying standardized data-
637 driven risk assessment, communication and risk-based control actions against *Campylobacter spp.*
638 across the European poultry meat chains using NS data.

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640 **Acknowledgements**

641 We are thankful to the French Ministry of Agriculture which funded the research activities with
642 the PhD grant of Francis Lauriau.

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644 **Conflict of interest:** None

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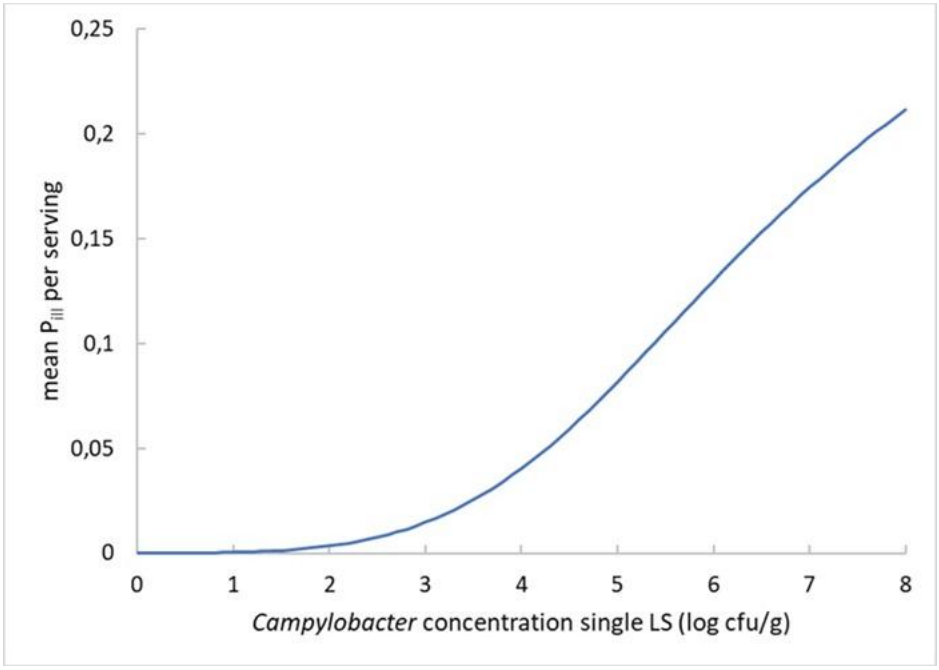
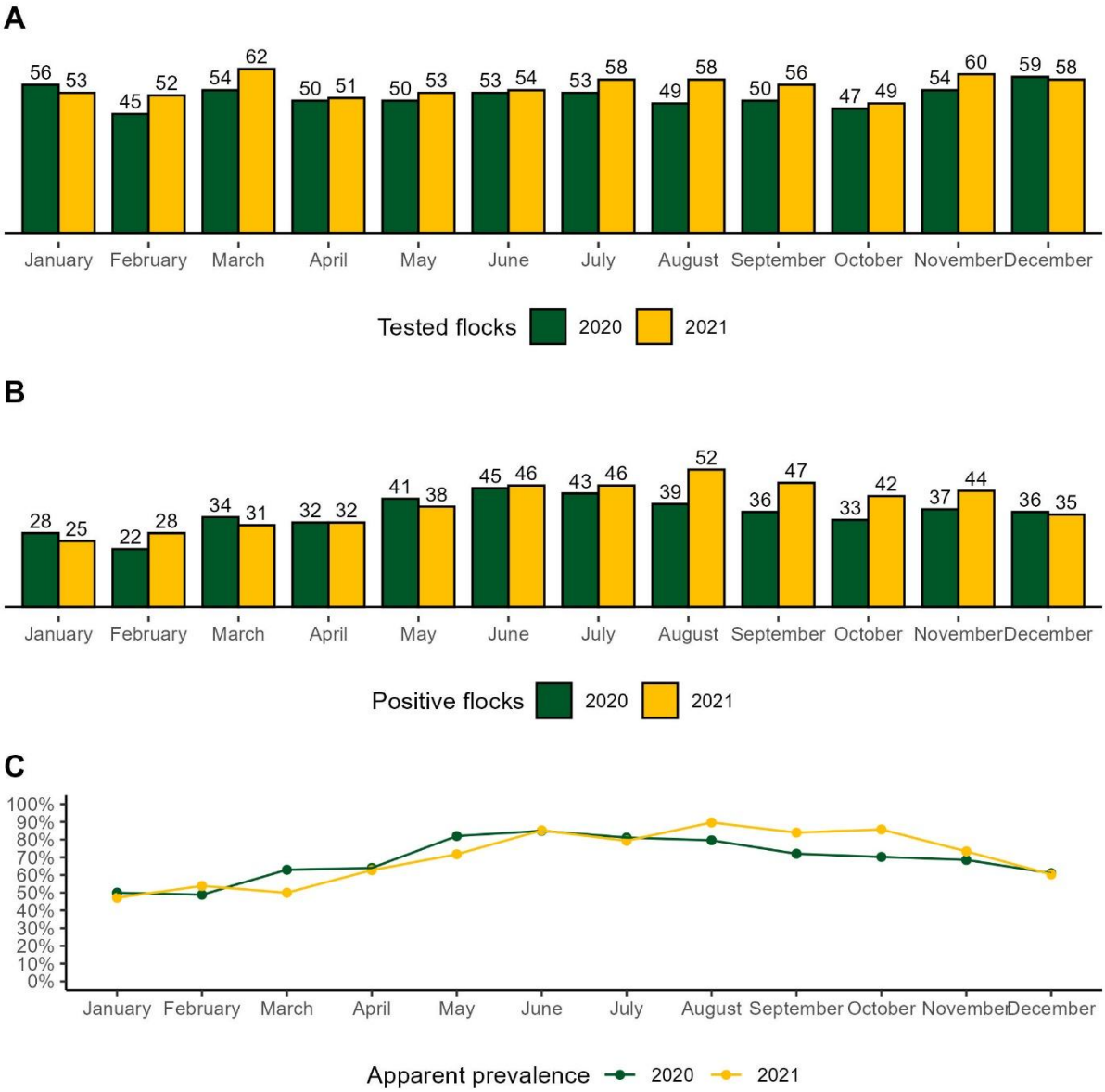
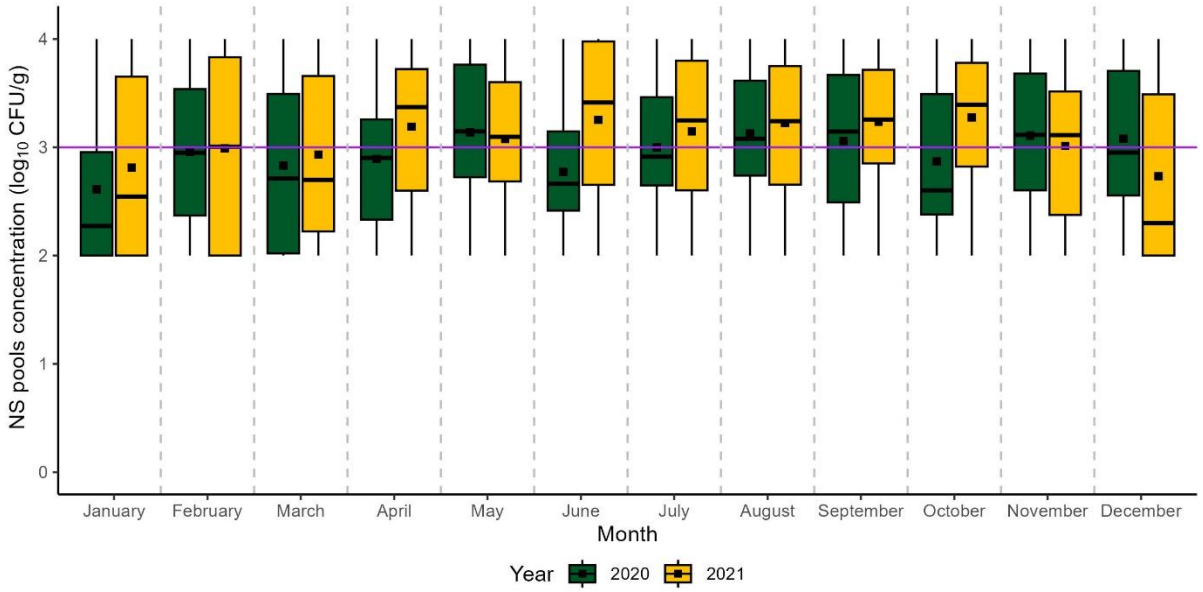


Figure 1. *Graphical representation of the relationship between Campylobacter concentrations found on single leg skin (LS) samples (x-axis) and the mean probability of illness per serving (y-axis) obtained from the stochastic version of the QMRA model after 91×50,000 iterations in a Monte Carlo simulation (Nauta et al., 2012).*



780

781 ***Figure 2.*** *Descriptive statistics on French Campylobacter surveillance data 2020-2021, based on testing of broiler*
782 *neck skin (NS) pools, standardized across slaughterhouses as described in Section 2.2 (values <100 set = 0 and values*
783 *>10,000 set = 10,000.* ***A)*** *Monthly number of tested flocks per year,* ***B)*** *Monthly number of NS positive flocks*
784 *(randomly sampled pool ≥ 100 CFU/g) per year,* ***C)*** *Monthly apparent prevalence (AP) per year.*



785

786 ***Figure 3.*** *Boxplots comparing the distributions of concentrations across randomly selected and positive (>100*
787 *CFU/g) NS pools (one pool per flock) across months of years 2020 and 2021. NB. Boxplots are constrained between*
788 *the minimum and maximum limits of* $log_{10} = 2$ *and* $log_{10} = 4$*, as explained in Section 2.2. The violet horizontal line*
789 *shows the limit of 1,000 CFU/g (i.e.* $log_{10} = 3$*) set in the European legislation and the black square on each boxplot*
790 *the mean* log_{10} *concentration.*

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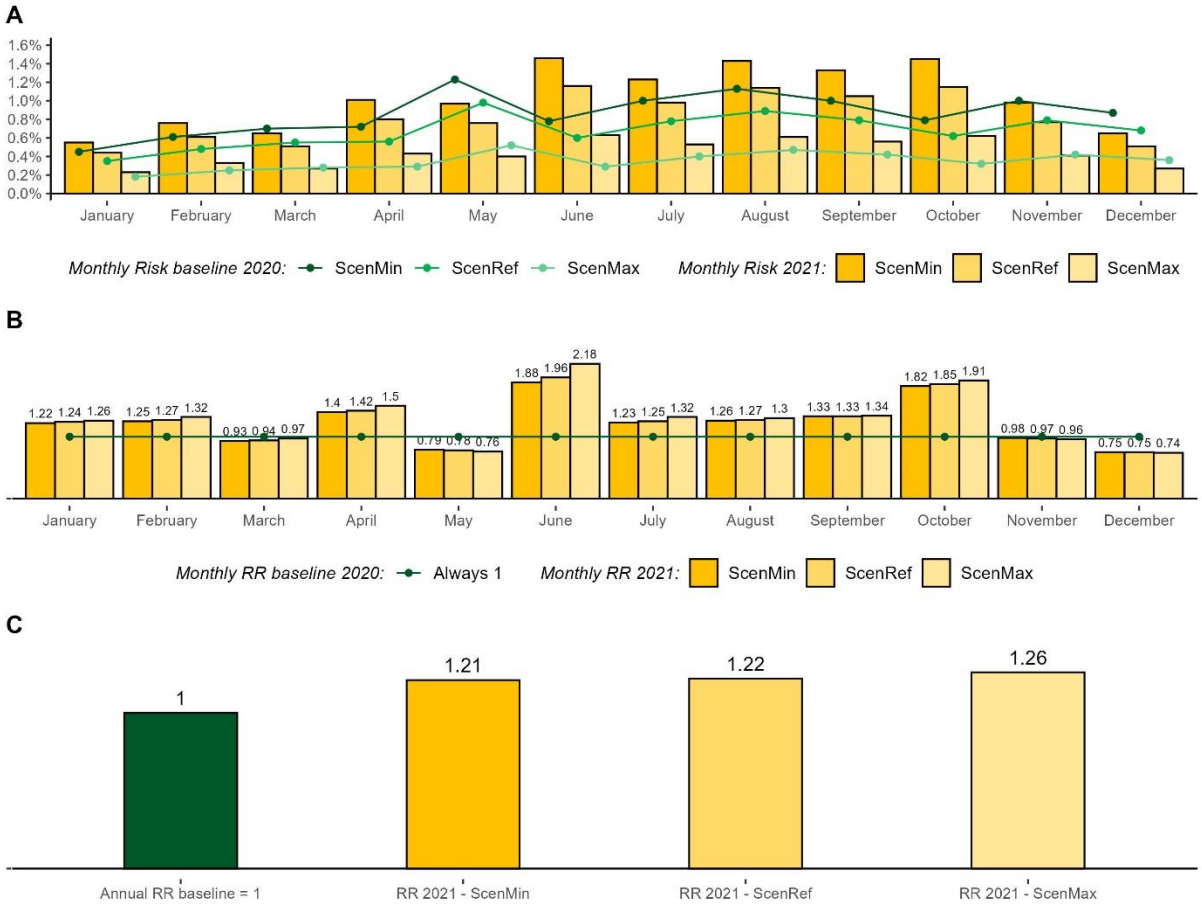
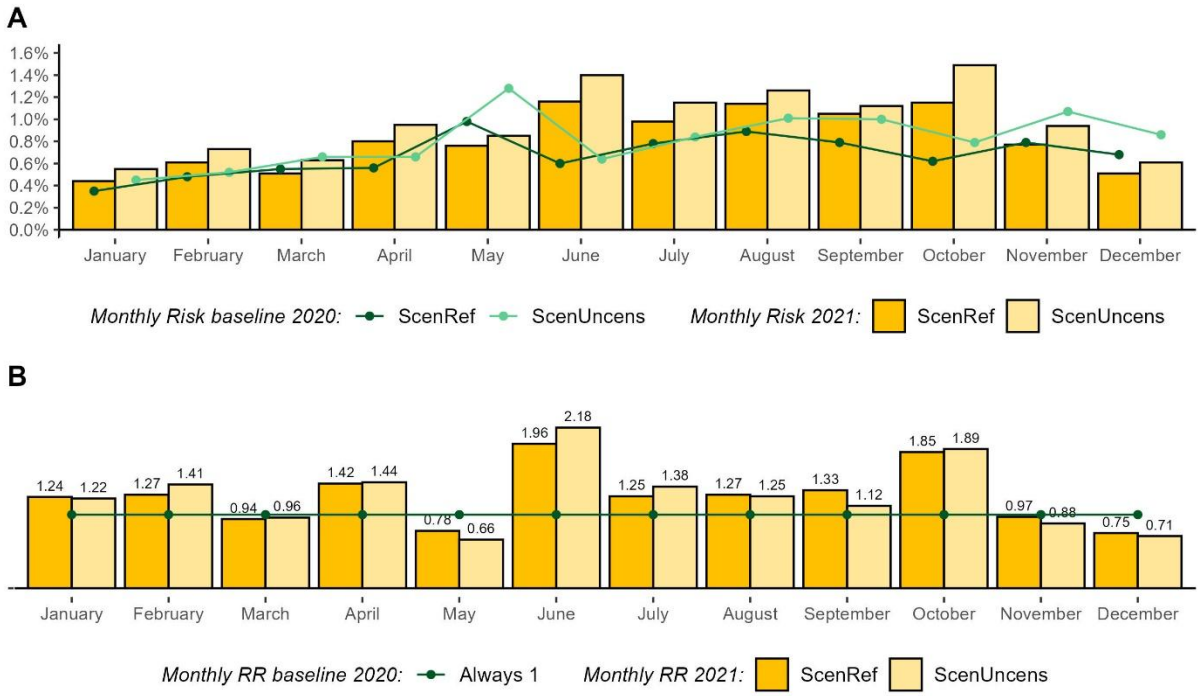


Figure 4. *Outputs of quantitative risk assessment according to year and simulation scenario (ScenMin with concentration transformation factor or CTF = 2; ScenRef with CTF = 3.2 and ScenMax with CTF= 10).* ***A)*** *Monthly absolute risk,* ***B)*** *Monthly relative risk (RR),* ***C)*** *Overall annual RR. NB. For 2020, the monthly and annual RRs are = 1, because this was the year used as reference (i.e. baseline).*



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798 ***Figure 5.*** *Outputs of quantitative risk assessment according to simulation scenario and year, to compare the reference*
799 *simulation scenario (ScenRef) and the scenario with censored data simulated (ScenUncens).* ***A)*** *Monthly absolute risk,*
800 ***B)*** *Monthly relative risk (RR). NB For 2020, the monthly RR equals to 1, because this was the year used as reference.*

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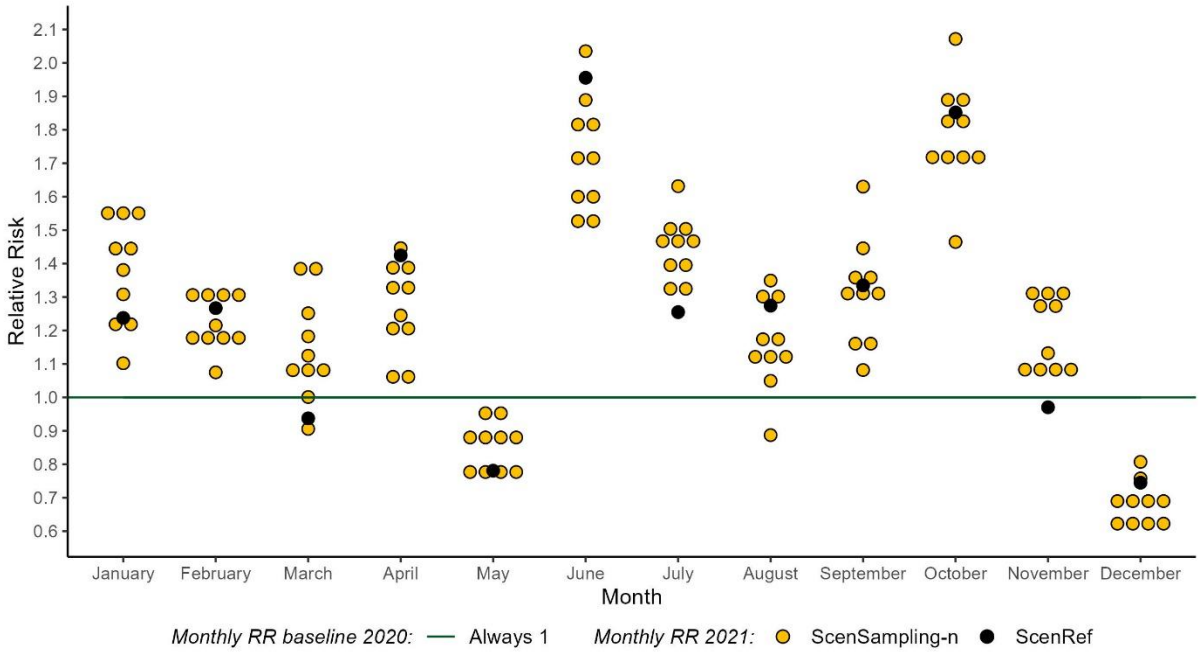

Figure 6. *Monthly relative risk (RR) according to ten simulation scenarios with CTF = 3.2. For 2020, the monthly RR equals to 1, because this was the year used as reference. For 2021, each monthly RR (ScenSampling1 to ScenSampling-10) is represented by a yellow dot, while the monthly RR of the reference scenario (ScenRef) is shown by a black dot.*