Food Control 145 (2023) 109471

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Survival of *Campylobacter jejuni* in frozen chicken meat and risks associated with handling contaminated chicken in the kitchen

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ARTICLE INFO

Keywords:
Campylobacter jejuni
ST-257
ST-918
Cross-contamination
Chicken meat
Kitchen

ABSTRACT

Most *Campylobacter* infections in humans are sporadic cases, often connected to private households. Chicken meat is believed to be the main source of human exposure to *Campylobacter* and there are significant risks of cross-contamination when handling *Campylobacter*-contaminated chicken in the kitchen. One post-harvest preventive measure to reduce *Campylobacter* concentrations on chicken meat is freezing. This study examined survival of different sequence types of *C. jejuni* during freezing and risk factors during handling of *C. jejuni*–contaminated chicken meat in the kitchen. Chicken fillets were artificially contaminated before freezing with two different sequence types of *C. jejuni* (ST-257 and ST-918), at concentrations in the meat of 4.1 $\log_{10}$ CFU/g (low) and 5.3 $\log_{10}$ CFU/g (high). Risk factors in the kitchen were assessed by swabbing gloves before and after washing, to simulate hands before and after washing. Utensils such as scissors and forceps used for cutting were also sampled, while a cutting board was sampled twice to simulate before and after wiping.

The greatest decrease in *Campylobacter* concentrations in the freezer occurred in the first four days and the decrease then flattened off. After 49 days in the freezer, concentrations on meat contaminated with high and low levels of ST-257 decreased by 2.0 $\log_{10}$ CFU/g and 1.5 $\log_{10}$ CFU/g, respectively, while concentrations on chicken meat contaminated with a high and low level of ST-918 decreased by 1.0 $\log_{10}$ CFU/g and 0.7 $\log_{10}$ CFU/g, respectively. *Campylobacter* was isolated from all simulated environmental samples. The highest load in the environment of both sequence types was unwashed gloves and the first sampling of the unwiped cutting board. Transfer from gloves and the cutting board was lower after washing/wiping, but high concentrations ($\geq$2 $\log_{10}$ CFU/mL rinse fluid) of *Campylobacter* persisted for all samples contaminated with ST-918 and for 18 of 20 samples contaminated with ST-257.

In conclusion, there are differences between *Campylobacter* sequence types in their ability to withstand freezing stress and *Campylobacter* remaining on hands after washing and on cutting boards after wiping is a likely source of cross-contamination in the kitchen.

1. Introduction

Campylobacter spp. is the most reported bacterial cause of gastrointestinal disease in humans in Europe and many other parts of the world (Center for Disease Control and Prevention, 2021; EFSA 2021; World Health Organization, ? 2015). Undercooking of chicken meat and cross-contamination during food preparation are critical factors for campylobacteriosis in humans. Contaminated chicken meat can act as a vehicle for *Campylobacter*, which can easily spread to kitchen equipment such as cutting boards, plates, and knives. Other possible transmission route are hands that handles the chicken meat and then touch the lips or food that should not be heated. Without further bactericidal treatment, food contaminated with *Campylobacter* can then cause human infection. A study of European consumers found that some assessed the cooking end-point of chicken meat based on its inner color or texture, a method that does not ensure inactivation of pathogens, and some were found to prioritize the juiciness of cooked chicken above any safety concerns (Langsrud et al., 2020).

To reduce the risk of exposure to *Campylobacter* at consumer level, different preventive measures can be adopted. According to a 2011

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https://doi.org/10.1016/j.foodcont.2022.109471
Received 10 June 2022; Received in revised form 18 October 2022; Accepted 19 October 2022
Available online 27 October 2022
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report by the EFSA's Panel of Biological Hazards, an estimated public health reduction of >50% can be achieved if all broiler batches comply with a limit of 1000 CFU/g, and a risk reduction of >90% if all batches comply with a limit of 500 CFU/g (EFSA, 2011). A later report estimated that a 3 $\log_{10}$ reduction in cecal concentrations would lead to a risk reduction of 50% for human campylobacteriosis connected to chicken meat (Koutsoumanis et al., 2020). A microbiological criterion for foodstuffs was introduced in 2005 for pathogens like *Salmonella* and *Listeria monocytogenes* (European Commission, 2005). Thirteen years later, *Campylobacter* was added and since 2018, the microbiological process hygiene criterion (PHC) for *Campylobacter* in chicken is set by European Commission regulation No. 2017/1495 (2018). The PHC aims to limit the number of bacteria in the food chain, improve food safety, and reduce cases of human campylobacteriosis linked to handling or consumption of chicken meat. If the PHC target is exceeded, the slaughterhouse must take action (EFSA & ECDC, 2021).

Freezing is effective in reducing *Campylobacter* counts and thereby reducing the risk of campylobacteriosis in humans (Georgsson et al., 2006). Formation of ice crystals, ice nucleation, and dehydration during freezing are factors that injure the bacteria (Alter & Reich, 2021). According to EFSA (2011), scientific opinion states that freezing for a few days reduces the concentration of *C. jejuni* in chicken meat by 0.9–1.4 $\log_{10}$ CFU/g and freezing for three weeks gives a reduction of 1.8–2.2 $\log_{10}$ CFU/g. A reduction of 0.6–2.9 $\log_{10}$ CFU/g on naturally contaminated carcasses after freezing and storing for 31 days and a reduction of 1 $\log_{10}$ CFU/g after one day were found in an Icelandic study (Georgsson et al., 2006). A Belgian study also found a 1 $\log_{10}$ CFU/g reduction for naturally contaminated meat after one day in the freezer, but thereafter the reduction was not significant (Sampers et al., 2010).

Over the years, multiple risk assessments have been made and theoretical models of cross-contamination and *Campylobacter* transfer rate have been created (Habib et al., 2020; Kusumaningrum et al., 2004; Lindqvist & Lindblad, 2008; Uyttendaele et al., 2006). Practical studies examining how *Campylobacter* is transferred from chicken meat to kitchen equipment have concluded that there is a significant cross-contamination risk when handling *Campylobacter*-contaminated chicken (Bai et al., 2020; Cardoso et al., 2021; Luber et al., 2006). Despite these numerous studies, there are still knowledge gaps regarding cross-contamination and transmission of *Campylobacter* in conjunction with normal kitchen routines. Furthermore, there are crucial gaps in consumer knowledge regarding storage temperatures, food pathogens, reheating, cleaning, and handling of risk foods (Lange et al., 2016; Marklinder et al., 2004; Marklinder et al., 2013). Underestimation of the level of contamination, together with poor hygiene practices, can lead to more cases of campylobacteriosis. Domestic kitchen practices are of the utmost importance when *Campylobacter* is introduced into the kitchen environment (Langsrud et al., 2020).

The aim of this study was to establish whether there are differences in survival between different sequence types of *C. jejuni* in chicken meat during freezing and whether the initial number of bacteria before freezing influence the amount of *Campylobacter* after thawing. A further aim was to determine the importance of different risk factors for consumers when handling *Campylobacter*-contaminated chicken meat in the kitchen.

2. Material and methods

2.1. Bacterial isolate

Two sequence types (ST) of *C. jejuni*, ST-257 and ST-918, were selected for use. The ST-257 was isolated previously by dead-end ultrafiltration from water pipes in a broiler house with broilers colonized by ST-257 during several rotations (Ferrari et al., 2019), while ST-918 was isolated from swab samples from transport crates after cleaning and disinfection (Frosth et al., 2020). These sequence types were chosen due to their connection with human campylobacteriosis, since both have previously been isolated from humans with campylobacteriosis (Public Health Agency of Sweden, 2017).

2.2. Sample preparation and quality control

Frozen chicken breast fillets without skin from conventionally produced Swedish chicken were purchased from a grocery store in Sweden, and thawed in a refrigerator for 24 h. A test according to ISO 10272 part 2 (ISO, 2017) was performed to ensure that the chicken breast fillets were not naturally contaminated with *Campylobacter*. This was done by taking 10 g meat from the surface of several fillets, placing it in a stomacher bag together with 90 mL Bolton enrichment broth (Oxoid, Basingstoke, UK), homogenizing the mixture, and incubation at 41.5 °C ± 0.5 °C for 44 ± 4 h in a microaerobic atmosphere generated by the use of CampyGen™ (Oxoid, Basingstoke, UK). The enriched culture was plated on modified charcoal cephoperazone desoxycholate agar (mCCDA) (Oxoid, Basingstoke, UK) and incubated at 41.5 °C ± 0.5 °C for 44 ± 4 h in a microaerobic atmosphere. All packages of fillets tested negative for thermotolerant *Campylobacter*.

For the experiment, the thawed chicken breast fillets were cut into pieces of approximately 50 g and placed in buckets together with their meat juice and 2 L of buffered peptone water (BPW). For the high concentration 45 ml of an overnight culture of *C. jejuni* ST-257 or ST-918 were mixed with the BPW before adding in the bucket with chicken breast fillets (Fig. 1). The same procedure was applied for the low concentration except that 1 ml of the overnight culture were used. After this preparation the bucket with pieces of chicken fillet, and the suspension of meat juice, BPW and *Campylobacter* broth were left 1 h at room temperature. Thereafter, each piece of 50 g breast fillet and 5 mL of the meat juice suspension were placed in separate stomacher bags and stored at −18 °C until analysis except five samples from each concentration and sequence type that were analyzed according 2.4. for quantification of the initial amount (day 0) of *Campylobacter* in the chicken breast fillets.

2.3. Simulated handling of chicken meat in the kitchen

The risk objects present in a kitchen environment (hands, well-used cutting board, utensils) were used for sampling in the laboratory. Five environmental samples were analyzed: nitrile gloves tested before and after rinsing in tap water (to simulate hands before and after washing); a well-used plastic cutting board, tested before (first) and after (second) wiping; and kitchen utensils. Sampling was performed on singles on these five types of sample and on 20 occasions, giving 20 samples per environmental sample and a total of 100 samples per *Campylobacter* sequence type.

The sampling of gloves before rinsing (simulated unwashed hands) was performed after a piece of chicken meat was picked up in a gloved hand, put down, and picked up once again. The glove was then removed from the hand and placed in the stomacher bag. The sampling of gloves after rinsing (simulated sloppy washed hand) followed the same procedure, but after the glove had been rinsed under running tap water of room temperature for 3–5 s before removal. In the first and second samplings of the plastic cutting board, separate 5 cm × 4 cm pieces of pristine Wettex dishcloth were used to swab the contaminated area of the board. Before use, the Wettex dishcloth was soaked in cold tap water, squeezed hard once, and then used for sampling. Another piece of Wettex dishcloth was used to swab the contact area of utensils (scissors and forceps) recently used for cutting the chicken sample. Each piece of dishcloth was then placed in a separate stomacher bag and used for quantitative and qualitative analysis.

2.4. Quantitative analysis

Five pieces of contaminated breast fillet were taken from the freezer and placed in refrigerator to thaw overnight before analysis. On the day

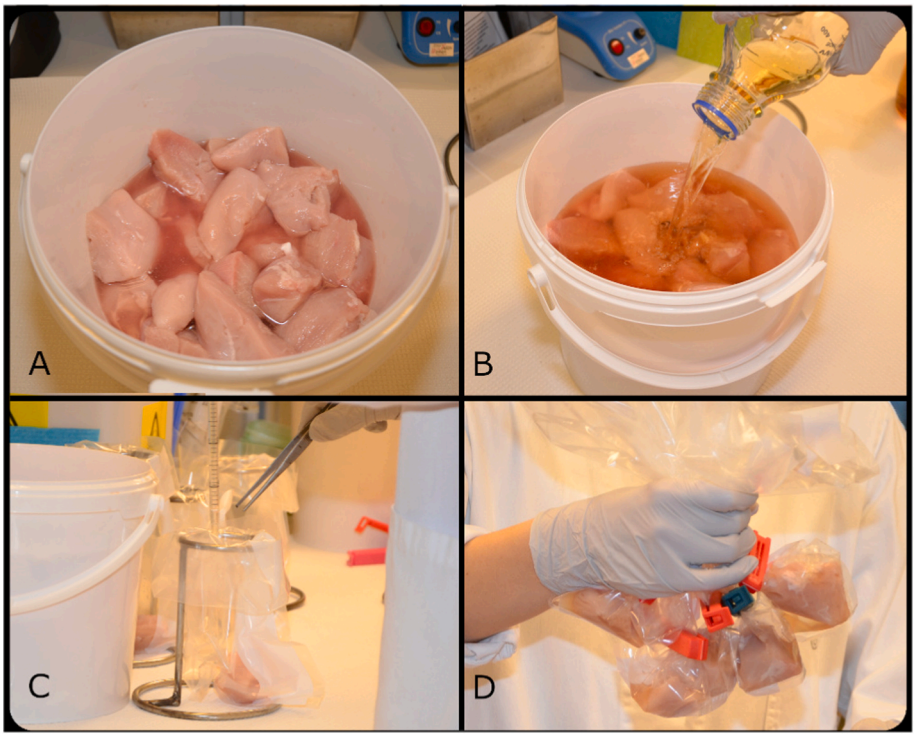

Fig. 1. Pieces of chicken breast fillet with meat juice in a bucket (A) together with BPW and overnight growth of *C. jejuni* (B). After preparation, each piece of chicken fillet were placed in a stomacher bag together with 5 ml of the meat juice-BPW suspension (C). Five samples from each concentration and sequence type (D) were analysed ten times during seven weeks.

of analysis, a 10 g sample from the surface of the breast fillet was excised by the same person throughout the experiment with the aim that sampling should be performed as similar as possible. The sample of breast fillet was placed in a stomacher bag together with 90 mL BPW. In the analysis of meat juice, 10 mL of the suspension was placed in a stomacher bag together with 90 mL BPW to yield a 1/10 dilution. For quantitative analysis of each environmental sample, 10 mL of BPW were added to each stomacher bag containing a nitrile glove or piece of Wettex dishcloth. The quantitative analysis of all samples was performed according to ISO 10272 part 2 (ISO, 2017). Briefly, the samples were homogenized in a stomacher (easyMIX Lab Blender, AES-Chemunex, Weber Scientific, Hamilton, New Jersey, USA) for 1 min at 240 rpm. A 10-fold serial dilution in 0.1% (v/v) peptone water (Dilucups, LabRobot Products AB, Stenungsund, Sweden) was prepared and 0.1 ml each from dilution 10^{-1}-10^{-3} were plated onto mCCDA. To detect low numbers of *Campylobacter*, 1.0 ml of the initial suspension was distributed on three plates of mCCDA. The plates were incubated at 41.5 °C ± 0.5 °C for 44 ± 4 h in a microaerobic atmosphere. After incubation, colonies characteristic of *C. jejuni* were quantified and the number in meat was expressed as $\log_{10}$ CFU/g, whereas the concentration in meat juice, and the suspensions from the environmental samples as $\log_{10}$ CFU/mL. The detection limit for meat juice was 1.0 CFU/mL and that for meat was expressed as 1.0 CFU/g.

2.5. Qualitative analysis

Qualitative analysis was carried out following enrichment of each environmental sample. For this, 90 mL Bolton broth were added to each stomacher bag containing glove/dishcloth and the bags were incubated at 41.5 °C in a microaerophilic atmosphere for 44 ± 4 h. If the quantitative analysis gave values below the detection limit of 1 $\log_{10}$ CFU/mL, the corresponding enriched culture was plated on mCCDA and incubated at 41.5 °C in a microaerophilic atmosphere for 44 ± 4 h. Samples below the detection limit, but detected after enrichment, are reported as (0 + detection limit)/2, resulting in 0.7 $\log_{10}$ CFU/mL.

2.6. Statistical analysis

The reduction in *Campylobacter* concentrations over time was studied by non-linear regression using a three-parameter exponential decay model as implemented in the R package drc (R Core Team, 2022; Ritz et al., 2015). When investigating the reduction during the first week, conventional multiple linear regression was performed, including interaction terms for time and concentration (high and low). All bacterial counts (CFU/g and CFU/mL) were $\log_{10}$-transformed and the fitting were performed on log-transformed values. However, the percentage of the reduction of bacteria were calculated on absolute values.

3. Results

3.1. Chicken fillet and meat juice

The reduction over time in *C. jejuni* ST-257 and ST-918 concentrations on chicken meat is shown in Figs. 2 and 3, respectively, together with the curves fitted with non-linear regression. As expected, the concentration of both ST-257 and ST-918 was reduced in the freezer (−18 °C), with the rate of reduction being greatest during the first four days of freezing for both *C. jejuni* sequence types. The concentration of *C. jejuni* decreased to a lesser extent during subsequent storage in the freezer. Overall, a significant difference ($p = 0.0001$) was found between the sequence types, with the reduction per day in days 0–7 being higher for ST-257 than ST-918, suggesting differences in their ability to survive freeze storage (Figs. 2 and 3).

The chicken meat contaminated with a high level of ST-257 had a mean concentration of 5.3 $\log_{10}$ CFU/g before freezing (day 0), whereas the chicken meat contaminated with a low concentration of ST-257 had a mean of 4.1 $\log_{10}$ CFU/g on day 0. The mean concentration of ST-918 on the chicken meat was almost identical to that of ST-257. After storage for 49 days, the decrease was 2.0 $\log_{10}$ CFU/g and 1.5 $\log_{10}$ CFU/g for the high and low concentration, respectively for ST-257. Whereas the concentration of *C. jejuni* ST-918 after 49 days storage in the freezer

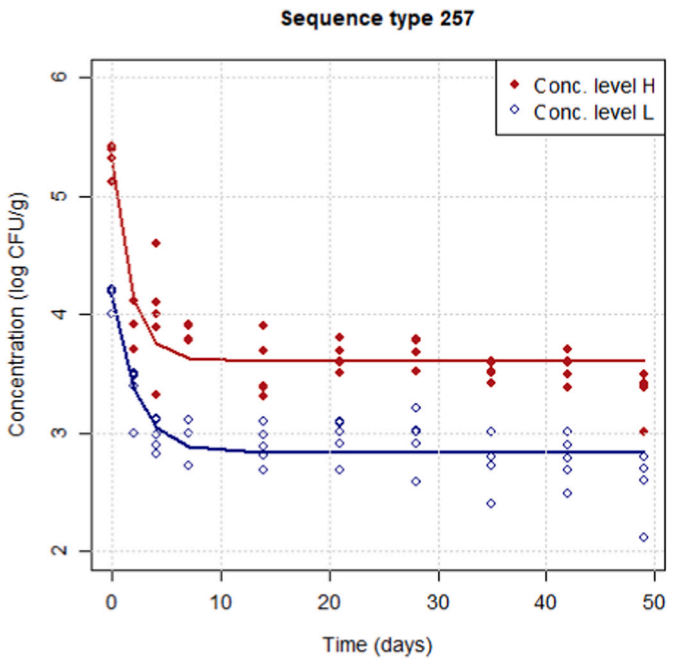

Fig. 2. Reduction in *C. jejuni* ST-257 concentration (log_{10} CFU/g) on artificially contaminated and deep-frozen chicken fillet analysed after thawing during the seven weeks (49 days) after thawing. H = high, L = low.

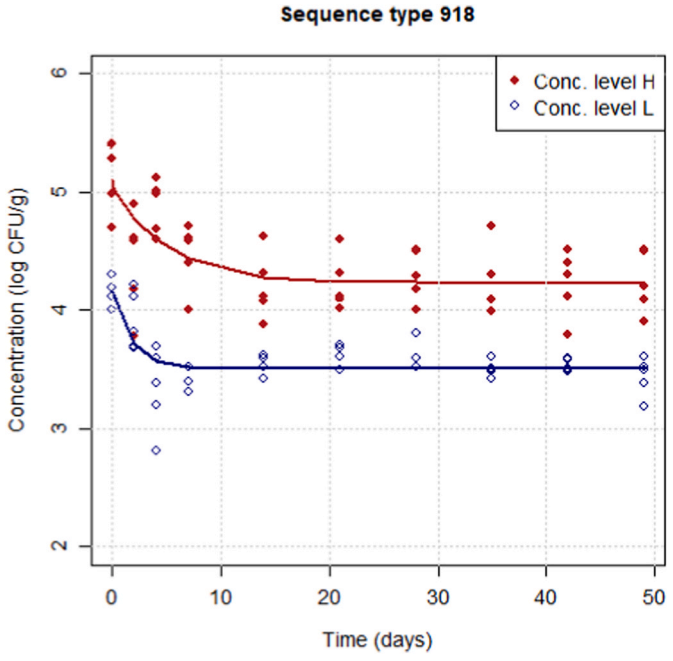

Fig. 3. Reduction in *C. jejuni* ST-918 concentration (log_{10} CFU/g) on artificially contaminated and deep-frozen chicken fillet analysed after thawing during the seven weeks (49 days) after thawing. H = high, L = low.

decreased by 1.0 log_{10} CFU/g and 0.7 log_{10} CFU/g on meat contaminated with a high and low concentration, respectively (Table 1). The mean initial concentration (day 0) of ST-257 in meat juice contaminated with a high and low concentration was identical (5.1 log_{10} CFU/mL). Whereas, the meat juice from chicken fillet contaminated with a high concentration of ST-918 contained 5.2 log_{10} CFU/mL on day 0 and the juice from meat contaminated with a low concentration 4.6 log_{10} CFU/mL on day 0. After 49 days in the freezer, the mean concentration in meat juice from thawed broiler meat contaminated with a high and low

Table 1
Concentration of *Campylobacter jejuni* in contaminated chicken breast fillet and their meat juice before and after seven weeks storage in the freezer.

Sample	Sequence type, concentration	Day 0, Mean (Range) log_{10} CFU/g	Day 49, Mean (Range) log_{10} CFU/g	Reduction log_{10} CFU/g	Reduction (%)[a]
Chicken	ST-257, High	5.3 (5.1–5.4)	3.3 (3.0–3.5)	2.0	98.9%
Fillet	ST-257, Low	4.1 (4.0–4.2)	2.6 (2.1–2.8)	1.5	96.8%
	ST-918, High	5.2 (4.7–5.4)	4.2 (3.9–4.5)	1.0	89.1%
	ST-918, Low	4.1 (4.0–4.3)	3.4 (3.2–3.6)	0.7	79.1%
Sample	Sequence type, concentration	Day 0, Mean (Range) log_{10} CFU/mL	Day 49, Mean (Range) log_{10} CFU/mL	Reduction log_{10} CFU/mL	Reduction (%)[a]
Meat	ST-257, High	5.1 (5.0–5.2)	3.2 (3.0–3.4)	1.9	98.8%
Juice	ST-257, Low	5.1 (5.0–5.2)	2.6 (2.4–2.7)	2.5	99.7%
	ST-918, High	5.2 (5.0–5.4)	3.9 (3.6–4.1)	1.3	95.3%
	ST-918, Low	4.6 (4.2–5.0)	3.0 (2.7–3.2)	1.6	97.7%

[a] *Note.* The percentage of the reduction are calculated on the absolute values and not on the log-transformed values.

level of *C. jejuni* ST-257 were 1.9 log_{10} CFU/mL and 2.5 log_{10} CFU/mL lower compared to day 0. Whereas, the total decrease of meat juice suspension from meat contaminated by with high or low concentration of ST-918 after 49 days in the freezer was 1.3 log_{10} CFU/mL and 1.6 log_{10} CFU/mL meat juice, respectively (Table 1). The difference between the sequence types in the amount of *C. jejuni* in chicken breast fillet and meat juice initially contaminated with a similar concentration of each sequence type (high or low) are shown in Fig. 4. This difference persisted regardless of whether a high or low level of contamination was used or whether chicken fillet or meat juice was sampled.

3.2. Handling of chicken meat in the kitchen

A significant positive relationship between the amount of bacteria on the meat and the amount of bacteria on kitchen surfaces was observed (Fig. 5). ST-257 was detected after enrichment of all samples, including the four out of the 20 samples where the amount of *Campylobacter* was below the detection limit for quantification. Two of those samples were from the utensils and two from the second sampling of the cutting board. ST-918 was also isolated after enrichment from all samples and was quantified in 18 of the 20 samples, with two samples from utensils being below the detection limit. The highest numbers of *Campylobacter* quantified (log_{10} CFU/mL) in the rinse fluid from the environmental samples for both sequence types were in samples from gloves simulating hands before rinsing and in the first samples from the cutting board. In some cases, there was a higher concentration of bacteria on the sampled surface than in the meat. Whereas second sampling of the cutting boards and sampling of utensils corresponded with the lowest concentration of both sequence types (Fig. 5). Regardless of sequence type there were significant differences in the amount of bacteria ($p < 0.05$) between gloves before and after rinsing, and between first and second time of wiping the cutting board. Although the amount of *Campylobacter* was significantly lower after rinsing the gloves, there was a remarkably large part of *Campylobacter* left after rinsing. A mean of 26% of the absolute number of *Campylobacter* could be quantified on the gloves after rinsing. A difference was found between the different sequence types where 32%

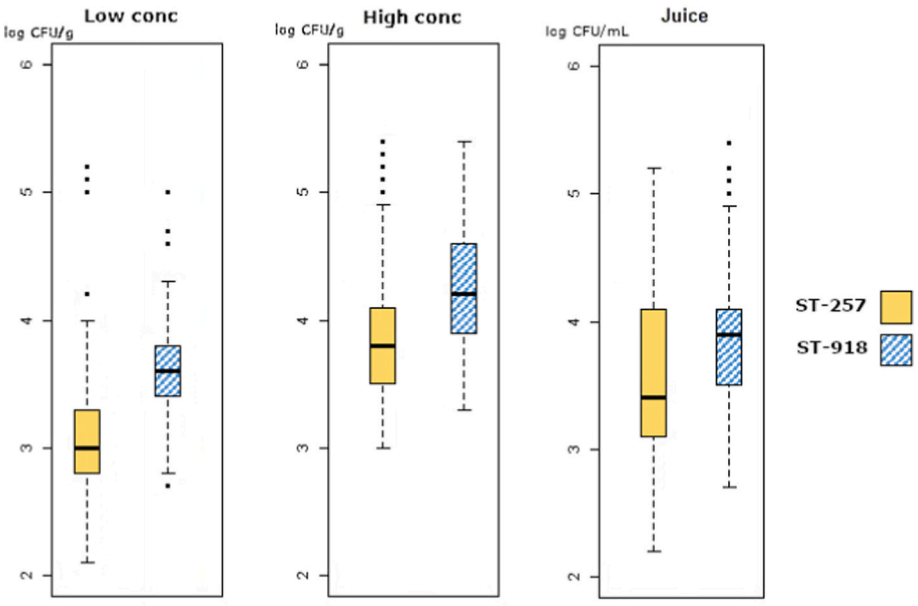

Fig. 4. Concentration of *C. jejuni* in chicken breast fillet (log_{10} CFU/g) initial contaminated of the same concentration of ST-257 or ST-918 low or high concentration and meat juice (log_{10} CFU/mL) from the 200 meat samples analysed after thawing.

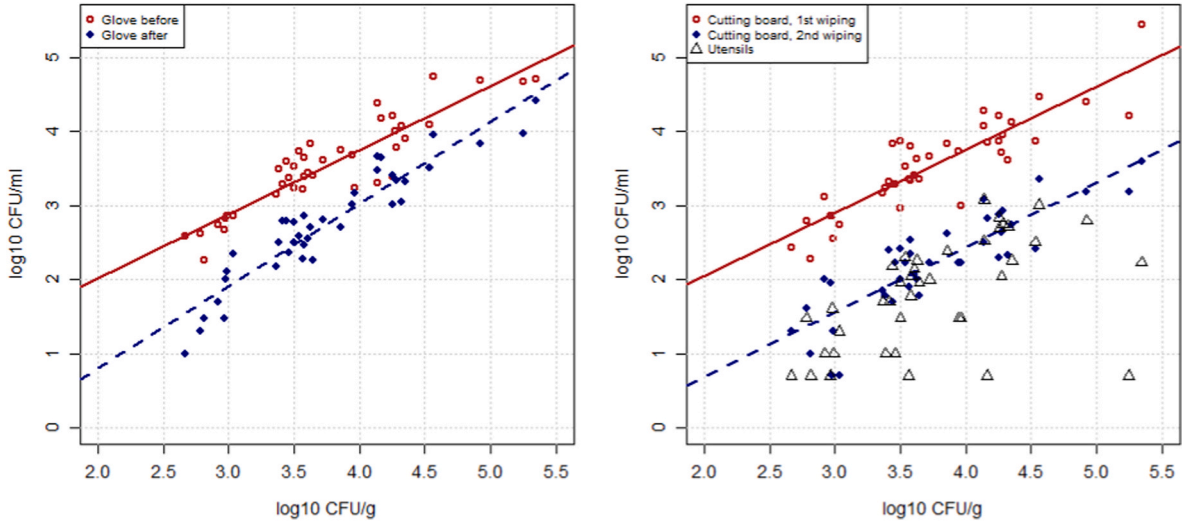

Fig. 5. Concentration of *C. jejuni* (log_{10} CFU /ml) in swab samples, in relation to the level of *C. jejuni* (log_{10} CFU/g) on the handled broiler meat, for gloves before and after washing, cutting board wiped first and second time, and utensils.

from ST-918 could be quantified from the gloves and 20% of ST-257. On the cutting board a mean of 6% of *Campylobacter* was left after wiping. However, there were no difference between the different sequence types regarding the concentration of *Campylobacter* left after wiping. On average, the rate of transfer was higher for ST-918 than for ST-257, regardless of the type of surface tested, but this difference was not statistically significant ($p = 0.20$). The slope was 0.98 for ST-257 and 0.72 for ST-918 (Fig. 6).

4. Discussion

There were clear differences in the rate of decrease in concentrations of the two *C. jejuni* types studied (ST-257, ST-918) after freezing of contaminated chicken meat. After 49 days of storage, the ST-257 concentration in the meat had decreased by 2.0 log_{10} CFU/g (98.9%) and 1.6 log_{10} CFU/g (96.8%) at the high and low levels of contamination, respectively, which corresponded to previous predictions (EFSA, 2011). However, the ST-918 concentration in the meat decreased by less, 1.0 log_{10} CFU/g (89.1%) and 0.7 log_{10} CFU/g (79.1%) for high and low levels of contamination after 49 days, which is lower than the reduction predicted by EFSA. This means that ST-918 survived to a greater extent in the freezer compared with ST-257. Previous studies investigating the reduction in *C. jejuni* on naturally and artificially contaminated meat generally also report a greater decline shortly after freezing, followed by a phase when the number of viable cells remains at stable levels (Georgsson et al., 2006; Ritz et al., 2007; Sampers et al., 2010; Sandberg et al., 2005), a pattern also observed in this study. Our results indicate that it is important to investigate the reduction at lower concentrations and not overestimate the reduction at high bacterial concentrations. The greater mean reduction after freezing observed at the high level of contamination corresponds to findings in a Danish study, where reductions of 1.2–1.8 from 7 log_{10} CFU/g and of 1.0–1.4 from 3 log_{10} CFU/g were recorded after seven days of freezing (Boysen et al., 2013).

The initial mean concentration of *C. jejuni* ranged from 4 to 5 log_{10}

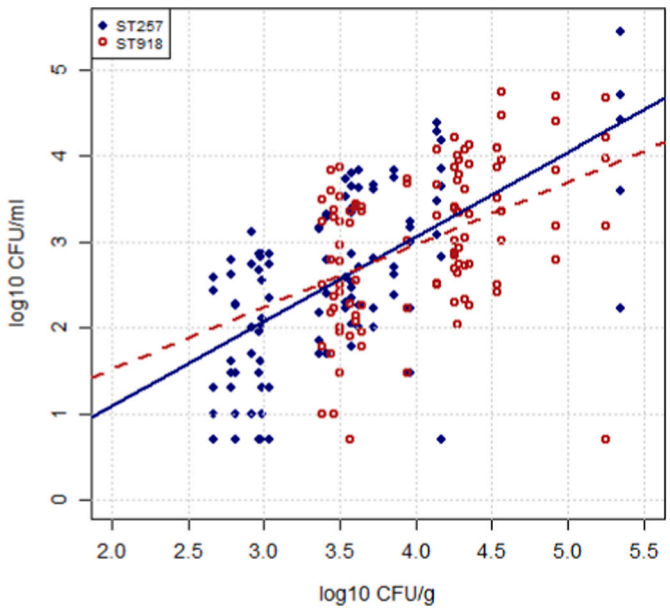

Fig. 6. Concentration of *C. jejuni* (log_{10} CFU/mL) on swab samples from all kitchen surfaces in relation to level of the two *C. jejuni* (log_{10} CFU/g) sequence types (ST-257, ST-918) on the handled broiler meat.

CFU for both meat and meat juice in this study. This initial concentration is slightly too high to reflect the level in Swedish chicken meat, which according to the EFSA baseline study is generally 2–3 log_{10} CFU/g (EFSA, 2010). However, an initial concentration of 4 log_{10} CFU/g was found in carcass skin sampled in the baseline study by other countries in Europe, with around 6% of samples from the 28 participating countries exceeding 4 log_{10} CFU/g, and around 16% showing counts of 3–4 log_{10} CFU/g (EFSA, 2010). In the recent EFSA publication reporting results from 2020 for 21 EU member states in the context of the *Campylobacter* PHC set out in Regulation (EC) No. 2073/2005, 17.8% of neck skin samples exceeded the limit of 3 log_{10} CFU/g (EFSA & ECDC, 2021). However, the extent to which the limit was exceeded was not stated.

The *C. jejuni* types ST-257 and ST-918 were chosen for this study due to their role in causing human campylobacteriosis. In an outbreak of campylobacteriosis in Sweden in 2016–2017 related to domestic broiler production, around 25% of sequenced isolates from patients were ST-918, while ST-257 was isolated from a few patients (Swedish Food Agency & Public Health Agency of Sweden, 2018). Moreover, ST-918 was the most frequently isolated sequence type in fresh retail chicken meat during investigations following the outbreak. However, ST-257 was the type most frequently isolated from hospital patients in Sweden in week 34 in 2019 (Public Health Agency of Sweden & Swedish Food Agency, 2020) and was significantly associated with hospitalization (Harvala et al., 2016).

In the present study, there was a numerical difference in bacterial transfer rate between ST-257 and ST-918, which might indicate a difference in their ability to adhere to different surfaces. Our theory is that ST-918 is more resistant to oxidative stress and has the ability to adhere and endure on surfaces in transport crates and at the slaughterhouse. Variations between sequence types in their tolerance to stresses, including freezing, increase the risk of human infection (Oh et al., 2018, 2019), so identification of differences in the ability to survive freezing and other physical tests is of interest. In future studies, comparing the results obtained for ST-257 and ST-918 with those for a ST not associated with human infection would be of interest, to determine the efficiency of freezing. The virulence factor for human infection might be related to the ability to withstand freezing, either due to high initial dose or in combination with other mechanisms of pathogenicity, such as the ability to attach to surfaces. Higher ability to survive stress might explain the Swedish outbreak in 2016, which was primarily caused by ST-918.

The within-kitchen transfer of *C. jejuni* documented in this study emphasizes the significant risk of cross-contamination when handling contaminated chicken meat. The transfer of ST-257 and ST-918 was shown to differ depending on environment and sequence type, and in some cases the concentration was higher in the environmental sample than on the contaminated chicken meat touched by that sample. Previous studies analyzing chicken meat have also found higher concentrations on the surface of the chicken compared with underlying muscle (Hansson et al., 2015). In the present study, chicken meat samples were collected from both internal and external parts of the chicken fillet, whereas only the external part was handled, and lower concentrations on samples of internal muscle could explain why some environmental samples had higher concentrations than the original chicken meat. Two samples from the cutting board were below the limit of quantification when cutting meat contaminated with ST-257, even though *Campylobacter* was quantified in the utensils used at the time. The most likely reason was that there were only a few bacteria left as only a low level was quantified on the utensils.

Campylobacter was quantified on all gloves after rinsing with water and mostly at high concentrations, with 35 of 40 samples having at least 2.0 log_{10} CFU/mL rinse fluid. Since *Campylobacter* should have been easier to remove by rinsing a smooth glove compared with bare hands, this indicates that hands are an important risk factor for transmission of *Campylobacter* and that washing thoroughly with antibacterial substances is of critical importance. The second sampling of the cutting board showed significantly lower transfer of *Campylobacter*, irrespective of the sequence type. However, there were still high levels of *Campylobacter* left on the cutting board after the first wiping, since the amount of *Campylobacter* from the second wiping was above the limit of detection (100 CFU/mL) for all samples contaminated with ST-918 and for 18 of 20 samples contaminated with ST-257. Thus if a cutting board remains unwashed, there is a likelihood of it cross-contaminating other foods (Habib et al., 2020). It is well known that even small failures in cleaning and cross-contamination can lead to human infection, since the infectious dose is low (Teunis et al., 2005; Verhoeff-Bakkenes et al., 2008). There may also be a difference in cross-contamination depending on the material of the cutting board, e.g., Bai et al. (2020) found that a plastic cutting board had statistically significant lower transfer rate than a wooden cutting board. This may be because plastic cutting boards are more often washed in dishwashers, i.e., for a longer time and at higher water temperature. In future studies, different cleaning scenarios for hands and kitchen equipment should be analyzed. Prolonging hand washing time or using a detergent on cutting boards might give lower transfer of *Campylobacter* to the equipment. As can be seen in Fig. 6, there was a clear difference in concentration of *Campylobacter* between the standard types used for cross-contamination in this study, because ST-257 showed a higher reduction in concentrations in the freezer. In future cross-contamination studies comparing different STs, it would be preferable to have similar concentrations of these STs on the meat handled by the environmental samples.

The first and second samplings of the cutting board and the sampling of utensils were performed using pristine Wettex dishcloth, to mimic the kitchen environment, but sterile cotton swabs could have been used instead. In previous studies, the domestic dishcloth has been shown to harbor significant concentrations of bacteria (Gillies, 2020; Hilton & Austin, 2000). Cardoso et al. (2021) isolated *Campylobacter* from a kitchen cloth and related this to unsafe handling practices in the kitchen. Utensils showed the lowest transfer of both sequence types in the present study but *Campylobacter* was isolated from 34 out of 40 samples tested, indicating a significant risk of cross-contamination when using small unwashed utensils. According to Kusumaningrum et al. (2003), *C. jejuni* can endure on stainless steel surfaces, but the lower transfer rate could be explained by the small surface area of stainless steel in scissors and forceps. This study has been focusing on the concentration of bacteria

but the risk of becoming infected by *Campylobacter* by cross-contamination in the kitchen is also depending on other factors such as the frequency of hands touching the face. Considering the low infection dose the risk of becoming infected by *Campylobacter* by direct transmission as contaminated hands touching the lips could probably be quite high. It is also important to consider the variation between e.g., aero-tolerant strains of *C. jejuni* (Oh et al., 2015). It is not common practice in private households to use scissors and forceps, which were suitable for laboratory work when handling raw chicken. To mimic the kitchen environment more closely, a kitchen knife with a larger contact area could be used instead. This might retain more meat juices than the scissors and forceps, allowing more extensive transfer of *Campylobacter*. A previous study on naturally *Campylobacter*-contaminated chicken breast fillets found that transfer rate of *Campylobacter* was sometimes greater to the knife than to the cutting board (Luber et al., 2006). This underscores the importance of adequate cleaning of used cutlery in preventing cross-contamination from *Campylobacter*-contaminated chicken, and thus preventing/reducing campylobacteriosis in humans.

5. Conclusions

Freezing reduced the concentrations of *C. jejuni* in chicken meat and meat juice, but did not eliminate the presence of all bacteria. The rate of decrease in *Campylobacter* concentrations on frozen chicken fillet was greatest in the first four days of freezing and flattened off thereafter. There was a difference in the ability of *C. jejuni* standard types to withstand the stress of freezing, with ST-918 decreasing to a lesser extent than ST-257, indicating a possible difference in their ability to cause disease. In the kitchen, the meat juice probably poses a greater risk than undercooked core meat, since the juice had similar concentrations of *C. jejuni* to the uncooked meat and the juice can easily spread to other surfaces in the kitchen.

Campylobacter was isolated from all environmental samples that touched contaminated chicken meat in the kitchen, with gloves (simulating hands) and cutting board posing the highest concentration, these must therefore be seen as major risk factors of transmission of *C. jejuni* from chicken meat to humans. High concentrations of *Campylobacter* were isolated from the gloves and cutting board even after washing/wiping. Good kitchen hygiene is thus of the utmost importance to prevent cross-contamination during handling of *Campylobacter*-contaminated chicken, and thus prevent or reduce the risk of campylobacteriosis in humans.

CRediT authorship contribution statement

Daniel Eriksson: Investigation, Data curation, Writing – original draft. **Ella Råhlén:** Investigation, Data curation, Writing – original draft. **Emma Bergenkvist:** Formal analysis, Supervision. **Moa Skarin:** Methodology. **Lise-Lotte Fernström:** Methodology. **Jesper Rydén:** Software, Formal analysis, Visualization. **Ingrid Hansson:** Conceptualization, Foundation, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

This work was supported by the Swedish Farmers' Foundation for Agricultural Research (SLF) [grant numbers O-18-20-158].

References

Alter, T., & Reich, F. (2021). Management strategies for prevention of *Campylobacter* infections through the poultry food chain: A European perspective. In S. Backert (Ed.), *Fighting Campylobacter infections* (pp. 79–102). Springer International Publishing. https://doi.org/10.1007/978-3-030-65481-8_4.

Bai, Y., Lin, X. H., Zhu, J. H., Cui, S. H., Guo, L. X., Yan, S. F., Wang, W., Xu, J., & Li, F. (2020). Quantification of cross-contamination of *Campylobacter jejuni* during food preparation in a model kitchen in China. *Journal of Food Protection*. https://doi.org/10.4315/JFP-20-280

Boysen, L., Wechter, N. S., & Rosenquist, H. (2013). Effects of decontamination at varying contamination levels of *Campylobacter jejuni* on broiler meat. *Poultry Science, 92*(5), 1425–1429. https://doi.org/10.3382/ps.2012-02889

Cardoso, M. J., Ferreira, V., Truninger, M., Maia, R., & Teixeira, P. (2021). Cross-contamination events of *Campylobacter* spp. in domestic kitchens associated with consumer handling practices of raw poultry. *International Journal of Food Microbiology, 338*, Article 108984. https://doi.org/10.1016/j.ijfoodmicro.2020.108984

Center for Disease Control and Prevention. (2021). *Campylobacter (Campylobacteriosis)*. Available from: . (Accessed 10 March 2022).

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, 8*(3). https://doi.org/10.2903/j.efsa.2010.1503

EFSA. (2011). 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, 9*(4), 2105. https://doi.org/10.2903/j.efsa.2011.2105, 2011.

EFSA and ECDC (European Food Safety Authority and European Centre for Disease Prevention and Control). (2021). The European union one health 2020 Zoonoses report. *EFSA Journal 2021, 19*(12), 324. https://doi.org/10.2903/j.efsa.2021.6971, 6971.

European Commission. (2005). Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. *Orkesterjournalen L*. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32005R2073. (Accessed 22 January 2021).

European Commission. (2018). COMMISSION REGULATION (EU) 2017/1495 of 23 August 2017 amending Regulation (EC) No 2073/2005 as regards *Campylobacter* in broiler carcases. https://eur-lex.europa.eu/eli/reg/2017/1495/oj. (Accessed 22 January 2021).

Ferrari, S., Frosth, S., Svensson, L., Fernström, L. L., Skarin, H., & Hansson, I. (2019). Detection of *Campylobacter* spp. in water by dead-end ultrafiltration and application at farm level. *Journal of Applied Microbiology, 127*(4), 1270–1279. https://doi.org/10.1111/jam.14379

Frosth, S., Karlsson-Lindsjö, O., Niazi, A., Fernström, L. L., & Hansson, I. (2020). Identification of transmission routes of *Campylobacter* and on-farm measures to reduce *Campylobacter* in chicken. *Pathogens, 9*(5). https://doi.org/10.3390/pathogens9050363

Georgsson, F., þorkelsson, Á. E., Geirsdóttir, M., Reiersen, J., & Stern, N. J. (2006). The influence of freezing and duration of storage on *Campylobacter* and indicator bacteria in broiler carcasses. *Food Microbiology, 23*(7), 677–683. https://doi.org/10.1016/j.fm.2005.10.003

Gillies, E. (2020). Determining the most effective common household disinfection method to reduce the microbial load on domestic dishcloths: A pilot study. *Environmental Health Review, 63*(4), 101–106. https://doi.org/10.5864/d2020-02

Habib, I., Coles, J., Fallows, M., & Goodchild, S. (2020). Human campylobacteriosis related to cross-contamination during handling of raw chicken meat: Application of quantitative risk assessment to guide intervention scenarios analysis in the Australian context. *International Journal of Food Microbiology, 332*, Article 108775. https://doi.org/10.1016/j.ijfoodmicro.2020.108775

Hansson, I., Nyman, A., Lahti, E., Gustafsson, P., & Olsson Engvall, E. (2015). Associations between *Campylobacter* levels on chicken skin, underlying muscle, caecum and packaged fillets. *Food Microbiology, 48*, 178–181. https://doi.org/10.1016/j.fm.2014.12.013

Harvala, H., Rosendal, T., Lahti, E., Engvall, E. O., Brytting, M., Wallensten, A., & Lindberg, A. (2016). Epidemiology of *Campylobacter jejuni* infections in Sweden, November 2011-October 2012: Is the severity of infection associated with *C. jejuni* sequence type? *Infection Ecology & Epidemiology, 6*, Article 31079. https://doi.org/10.3402/iee.v6.31079

Hilton, A. C., & Austin, E. (2000). The kitchen dishcloth as a source of and vehicle for foodborne pathogens in a domestic setting. *International Journal of Environmental Health Research, 10*(3), 257–261. https://doi.org/10.1080/09603120050127202

ISO. (2017). Microbiology of the food chain– Horizontal method for detection and enumeration of *Campylobacter* spp. – Part 1: Detection method—Part 2: Colony count technique, 2017 https://www.iso.org/standard/63228.html.

EFSA Panel on Biological Hazards, Koutsoumanis, K., Allende, A., Alvarez-Ordóñez, A., Bolton, D., Bover-Cid, S., Davies, R., De Cesare, A., Herman, L., Hilbert, F., Lindqvist, R., Nauta, M., Peixe, L., Ru, G., Simmons, M., Skandamis, P., Suffredini, E., Alter, T., Crotta, M., Ellis-Iversen, J., … Chemaly, M. (2020). Update and review of control options for *Campylobacter* in broilers at primary production. *EFSA Journal, 18*(4). https://doi.org/10.2903/j.efsa.2020.6090

Kusumaningrum, H. D., Riboldi, G., Hazeleger, W. C., & Beumer, R. R. (2003). Survival of foodborne pathogens on stainless steel surfaces and cross-contamination to foods. *International Journal of Food Microbiology, 85*(3), 227–236. https://doi.org/10.1016/s0168-1605(02)00540-8

Kusumaningrum, H. D., Van Asselt, E. D., Beumer, R. R., & Zwietering, M. H. (2004). A quantitative analysis of cross-contamination of *Salmonella* and *Campylobacter* spp.

via domestic kitchen surfaces. *Journal of Food Protecection, 67*(9), 1892–1903. https://doi.org/10.4315/0362-028X-67.9.1892, 2004.

Lange, M., Göranzon, H., & Marklinder, I. (2016). Self-reported food safety knowledge and behaviour among Home and Consumer Studies students. *Food Control, 67*, 265–272. https://doi.org/10.1016/j.foodcont.2016.03.014

Langsrud, S., Sørheim, O., Skuland, S. E., Almli, V. L., Jensen, M. R., Grøvlen, M. S., Ueland, Ø., & Møretrø, T. (2020). Cooking chicken at home: Common or recommended approaches to judge doneness may not assure sufficient inactivation of pathogens (Sant'Ana, A. de S., red.) *PLoS One, 15*(4), Article e0230928. https://doi.org/10.1371/journal.pone.0230928.

Lindqvist, R., & Lindblad, M. (2008). Quantitative risk assessment of thermophilic *Campylobacter* spp. and cross-contamination during handling of raw broiler chickens evaluating strategies at the producer level to reduce human campylobacteriosis in Sweden. *International Journal of Food Microbiology, 121*(1), 41–52. https://doi.org/10.1016/j.ijfoodmicro.2007.10.008

Luber, P., Brynestad, S., Topsch, D., Scherer, K., & Bartelt, E. (2006). Quantification of *Campylobacter* species cross-contamination during handling of contaminated fresh chicken parts in Kitchenscogan. *Applied and Environmental Microbiology, 72*(1), 66–70. https://doi.org/10.1128/AEM.72.1.66-70.2006

Marklinder, I. M., Lindblad, M., Eriksson, L. M., Finnson, A. M., & Lindqvist, R. (2004). Home storage temperatures and consumer handling of refrigerated foods in Sweden. *Journal of Food Protection, 67*(11), 2570–2577. https://doi.org/10.4315/0362-028x-67.11.2570

Marklinder, I., Magnusson, M., & Nydahl, M. (2013). CHANCE: A healthy lifestyle in terms of food handling and hygiene. *British Food Journal, 115*(2), 223–234. https://doi.org/10.1108/00070701311302203

Oh, E., Andrews, K. J., McMullen, L. M., & Jeon, B. (2019). Tolerance to stress conditions associated with food safety in *Campylobacter jejuni* strains isolated from retail raw chicken. *Scientific Reports, 9*(1), Article 11915. https://doi.org/10.1038/s41598-019-48373-0

Oh, E., Chui, L., Bae, J., Li, V., Ma, A., Mutschall, S. K., Taboada, E. N., McMullen, L. M., & Jeon, B. (2018). Frequent implication of Multistress-tolerant *Campylobacter jejuni* in human infections. *Emerging Infectious Diseases, 24*(6), 1037–1044. https://doi.org/10.3201/eid2406.171587

Oh, E., McMullen, L., & Jeon, B. (2015). High prevalence of Hyper-Aerotolerant *Campylobacter jejuni* in retail poultry with potential implication in human infection. *Frontiers in Microbiology, 6*, 1263. https://doi.org/10.3389/fmicb.2015.01263

Public Health Agency of Sweden & Swedish Food Agency. (2020). *Campylobacter från butik och klinik 2019*. https://www.livsmedelsverket.se/bestall-ladda-ner-material/sok-publikationer/artiklar/2019/s-2019-nr-01-campylobacter-fran-butik-och-klinik-livsmedelsverkets-rapportserie-s. (Accessed 30 April 2021).

Public Health Agency of Sweden. (2017). *Epidemiologisk typning av campylobacterisolat insamlade vecka 34 2017*. https://www.folkhalsomyndigheten.se/contentassets/bc4b2a860df24cec84568829414f6cd6/campylobacter-typningsrapport-v-34.pdf. (Accessed 9 May 2021).

R Core Team. (2022). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. URL https://www.R-project.org/.

Ritz, C., Baty, F., Streibig, J. C., & Gerhard, D. (2015). Dose-response analysis using R. *PLoS One, 10*(12), Article e0146021, 2015 https://doi: 10.1371/journal.pone.0146021.

Ritz, M., Nauta, M. J., Teunis, P. F. M., van Leusden, F., Federighi, M., & Havelaar, A. H. (2007). Modelling of *Campylobacter* survival in frozen chicken meat. *Journal of Applied Microbiology, 103*(3), 594–600. https://doi.org/10.1111/j.1365-2672.2007.03284.x

Sampers, I., Habib, I., De Zutter, L., Dumoulin, A., & Uyttendaele, M. (2010). Survival of *Campylobacter* spp. in poultry meat preparations subjected to freezing, refrigeration, minor salt concentration, and heat treatment. *International Journal of Food Microbiology, 137*(2–3), 147–153. https://doi.org/10.1016/j.ijfoodmicro.2009.11.013

Sandberg, M., Hofshagen, M., Østensvik, Ø., Skjerve, E., & Innocent, G. (2005). Survival of *Campylobacter* on frozen broiler carcasses as a function of time. *Journal of Food Protection, 68*(8), 1600–1605. https://doi.org/10.4315/0362-028X-68.8.1600

Swedish Food Agency & Public Health Agency of Sweden. (2018). *Campylobacter från butik och klinik – jämförelser under och efter utbrottet 2016–2017*. https://www.livsmedelsverket.se/bestall-ladda-ner-material/sok-publikationer/artiklar/2018/2018-nr-10. (Accessed 30 April 2021).

Teunis, P., van den Brandhof, W., Nauta, M., Wagenaar, J., van den Kerkhof, H., & van Pelt, W. (2005). A reconsideration of the *Campylobacter* dose-response relation. *Epidemiology and Infection, 133*(4), 583–592. https://doi.org/10.1017/S0950268805003912

Uyttendaele, M., Baert, K., Ghafir, Y., Daube, G., De Zutter, L., Herman, L., Dierick, K., Pierard, D., Dubois, J. J., Horion, B., & Debevere, J. (2006). Quantitative risk assessment of *Campylobacter* spp. in poultry based meat preparations as one of the factors to support the development of risk-based microbiological criteria in Belgium. *International Journal of Food Microbiology, 111*(2), 149–163. https://doi.org/10.1016/j.ijfoodmicro.2006.05.023

Verhoeff-Bakkenes, L., Beumer, R. R., de Jonge, R., van Leusden, F. M., & de Jong, A. E. I. (2008). Quantification of *Campylobacter jejuni* cross-contamination via hands, cutlery, and cutting board during preparation of a chicken fruit salad. *Journal of Food Protection, 71*(5), 1018–1022. https://doi.org/10.4315/0362-028x-71.5.1018

World Health Organization. (2015). *WHO estimates of the global burden of foodborne diseases: Foodborne disease burden epidemiology reference group 2007-2015*. World Health Organization. https://apps.who.int/iris/handle/10665/199350. (Accessed 22 January 2021).