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1 **Quantitative approach to assess the compliance to a performance objective (PO) of**
2 ***Campylobacter jejuni* in poultry meat in France**

3

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9

10 **Abstract**

11 Predictive modelling is used in microbiological risk assessment to quantify the growth and
12 inactivation of microorganisms through the use of mathematical models. *Campylobacter jejuni* is
13 one of the main foodborne pathogens and broiler meat is considered as the most important source
14 of human campylobacteriosis. The purpose of this study was to assess the effects of heating and
15 chilling during the poultry slaughter process on inactivation kinetics of *Campylobacter jejuni*
16 during chilled storage in order to predict its contamination level prior to preparation and
17 consumption in the consumer's home, and then to assess the compliance to a Performance
18 Objective (PO). Three strains of *C. jejuni* were submitted to consecutive heat (54°C for 3 min)
19 and cold (3°C for 2 h) stresses, mimicking the two main slaughtering steps, *i.e.* scalding and
20 chilling, by inoculating chicken fillets with three different concentrations (4, 6 and 8 log₁₀
21 CFU/g). Fillets were then stored at 6°C during 17 days under the modified atmosphere currently
22 used by food processors (70% O₂ / 30% CO₂). For all strains, bacterial log reduction was the
23 lowest when inoculated at 8 log₁₀ CFU/g. One strain showed an enhanced resistance during cold
24 storage after application of stressing steps, suggesting an impact of the cell history on further
25 bacterial resistance. Taking strain variability into account, after six days of storage, predictions
26 showed compliance of ready-to-be-cooked chicken meat with an hypothetical PO of 2.55 log₁₀
27 CFU/g, value set before the meat enters the consumer's home by the ICMSF (International
28 Commission on Microbiological Specifications for Foods). This study opens the path to assess
29 the compliance to a PO of *Campylobacter jejuni* in poultry meat and more generally provides
30 inputs to refine microbiological risk assessment by taking into account the cell history and more
31 particularly the impact of stressful steps on the subsequent inactivation at consumer's home.

32

33 **Keywords:** Foodborne pathogen; Food safety; Prediction; Slaughter process; Cell history

34 **1. Introduction**

35 All food-related companies need to ensure microbiological food safety (Regulation
36 "General Food Law" 178/2002). The implementation of prerequisite programs including Good
37 Hygiene Practices (GHP) and application of the Hazard Analysis and Critical Control Point
38 (HACCP) system are the main procedures to ensure food safety. In support of these measures,
39 Regulation EC No 2073/2005 of the European Parliament laid down microbiological criteria for
40 food. Approaches based on the estimation of the risk caused by microorganisms in the food chain
41 are part of the Microbiological Risk Assessment (MRA) described by the *Codex Alimentarius*
42 Commission (Codex Alimentarius Commission, Adopted 1999-Amendments 2012 and 2014).

43 MRA is a scientific discipline determining the public health risk associated with
44 biological hazards. This discipline is generally encouraged by national food safety organizations,
45 particularly to support decision making. However, this procedure is also of interest to
46 manufacturers, in particular for determining the shelf life of foodstuffs, process optimisation or
47 the development of new formulations. To assess microbiological risks, predictive microbiology is
48 a widely used procedure, especially since the early 2000s. It is a discipline that aims to quantify
49 the contamination level of microorganisms (following growth or inactivation) through the use of
50 mathematical models. Besides, predictive microbiology is a major tool in the design of HACCP
51 plans by identifying hazards and critical control points and specifying limits and corrective
52 actions (Miles and Ross, 1999; Ross and McMeekin, 1997).

53 Since 1999, the Codex Alimentarius provided guidelines for the conduct of
54 microbiological risk and recommended that industry and control authorities adopt new risk
55 management metrics referred to as Food Safety Objective (FSO), Performance Objective (PO),
56 and Performance Criterion (PC) (Codex Alimentarius Commission, Adopted 1999-Amendments
57 2012 and 2014; ICMSF, 2018). The ICMSF noted FSOs/POs as “the scientific basis for industry

58 to select and implement measures that control the hazard(s) of concern in specific foods or food
59 operations, for control authorities to develop and implement inspection procedures to assess the
60 adequacy of control measures adopted by industry, and for quantifying the equivalence of
61 inspection procedures in different countries”. However, distinction has to be made between PO
62 and FSO. A PO is “the maximum tolerable level of a relevant hazard in the food at a specific
63 point in the food supply chain. FSO is defined as “the maximum tolerable level of a relevant
64 hazard in the food at the point of consumption.”

65 In this context, industry and other stakeholders of the poultry sector are focused on the
66 foodborne pathogen *Campylobacter* that is the most common cause of bacterial gastroenteritis in
67 Europe in humans (EFSA and EDC, 2018). Largely under-estimated, in 2017 “the number of
68 reported confirmed cases of campylobacteriosis was 246,158 with an EU notification rate of 64.8
69 per 100,000 population” (EFSA and EDC, 2018). Usually, infection with *Campylobacter jejuni*
70 are self-limiting but in some cases, infection can lead to chronic sequelae, such as Guillain-Barré
71 syndrome (Nyati and Nyati, 2013).

72 The main reservoir of *Campylobacter* is poultry, and this pathogen is able to survive
73 slaughtering steps with a high prevalence and level of contamination in poultry carcasses (EFSA,
74 2010; EFSA and EDC, 2018). In addition, it was shown recently that in liquid cultures, stress
75 induced by heating or chilling steps have an impact on log reduction of *C. jejuni* during storage at
76 refrigerated temperature and modified atmosphere (Duqué et al., 2019). Several MRA have been
77 performed on *Campylobacter* to develop practical risk management strategies (ICMSF, 2018).
78 However, variability associated with the bacterial behavior, especially due to the steps previously
79 encountered by the bacteria, *i.e.* cell history, is not always considered, especially when using
80 experimental laboratory approaches. Indeed, it has been reported in other bacteria that exposure
81 to a stress could increase resistance to a subsequent stress, which might require new generation of

82 predictive models (Den Besten et al., 2010; Desriac et al., 2013). Several studies conducted on *C.*
83 *jejuni* also highlighted the impact of cell history on the pathogen behavior (Duqué et al., 2019;
84 Klančnik et al., 2009; Murphy et al., 2003a; Murphy et al., 2003b; Oh et al., 2015; Reid et al.,
85 2008); nevertheless these works were mainly performed on synthetic culture media, which
86 reinforces the need to take into account the cell history on *C. jejuni* behavior on food matrices. In
87 the current study, three strains of *C. jejuni* inoculated on chicken fillets at different
88 concentrations, were submitted to successive stresses close to those encountered during slaughter
89 (Duqué et al., 2019). The objective of this study was i) to assess the effect of poultry slaughter
90 process on the subsequent log reduction of *C. jejuni* during cold storage in order to predict the
91 contamination level prior to preparation and consumption in the consumer's home, ii) to assess
92 the compliance to a Performance Objective, for this purpose the value of 2.55 log₁₀ CFU/g before
93 the chicken enters the consumer's home, set by ICMSF was chosen as reference.

94

95 **2. Materials and methods**

96 *2.1 Bacterial strains and culture conditions*

97 The culture conditions used were described previously (Duqué et al., 2019). Briefly, three
98 strains from poultry origin were used: *C. jejuni* C09MJLT205, *C. jejuni* RM1221 and *C. jejuni*
99 C97anses 640 (Fouts et al., 2005; Guyard-Nicodeme et al., 2015; Guyard-Nicodeme et al., 2013).
100 Strains were previously selected according to their behavior under stressful conditions and their
101 ST-Clonal Complex in order to have **three distinct, but representative, strains to cover the**
102 **variability of** our collection. From cultures stored at -80°C in brain heart infusion (BHI,
103 Biomérieux, Marcy l'Etoile, France) supplemented with 20% (v/v) glycerol, *C. jejuni* strains
104 were cultured on Karmali (Oxoid, Dardilly, France) agar plates at 42°C for 48-72 h under

105 microaerobic conditions. Then, two successive cultures were incubated in Mueller-Hinton broth
106 (MH, Oxoid, Dardilly, France) for 20h and 18h under microaerobic atmosphere and under
107 agitation. Finally, a decimal dilution was carried out to obtain the final culture.

108

109 2.2 *Experimental procedure*

110 Chicken fillets without skin provided by a French poultry slaughterhouse were frozen at
111 -20°C before use. Thawed chicken fillet cuts of 10 g (± 1 g) were put in resealable plastic bags.
112 The pieces were surface inoculated with *C. jejuni* at a final concentration of 10^4 , 10^6 or 10^8
113 CFU/g by adding 0.5 mL of bacterial suspensions, respectively at 5, 7 or 9 \log_{10} CFU/mL directly
114 into the bag. To ensure that the inoculation was homogeneous, the bags were kneaded for a few
115 moments and allowed to stand for thirty minutes in order to facilitate the adherence of the
116 bacteria to the meat (Eideh and Al-Qadiri, 2011; Haughton et al., 2012; Meredith et al., 2013;
117 Nair et al., 2014). For each experiment, two control pieces were inoculated with sterile
118 physiological water to quantify *C. jejuni* cells naturally contaminating the chicken fillets.

119 Stresses mimicking scalding and chilling steps of chicken slaughtering process, considered
120 as stressful for *C. jejuni*, were applied as previously described (Duqué et al., 2019). Inoculated
121 chicken pieces were subjected to successive steps mimicking, at laboratory scale, scalding (heat
122 stress), chilling (cold stress) and the storage steps, according to a full experimental design. In
123 parallel, chicken fillets were subjected only to the storage step in order to be able to compare
124 stressed and unstressed cells. The chicken fillets were placed in plastic bags; **to avoid an isolating**
125 **layer of air slowing down the heat transfer, air expulsion was handmade.** Bags were immersed in
126 a water bath at 54°C for 3 minutes (assimilated to scalding), 54°C was chosen as it was the
127 maximal observed temperature of scalding baths during our visit of French chicken

128 slaughterhouses. Fillets were then cooled in a water bath for 5 min at 22°C, corresponding to the
129 ambient conditions that occur between scalding and chilling steps in slaughterhouse. In both
130 cases, the surface temperature was measured using a probe Kistock KTT220 (KIMO, Montpon,
131 France), the temperature was achieved within 10 s. Then, the chicken pieces were removed from
132 the bags and placed in Petri dish to be incubated in a refrigerated and ventilated incubator at 3°C
133 for 2 hours (assimilated to chilling) in order to maximize air circulation. The temperature of 3°C
134 was chosen because it represents the targeted core temperature of poultry carcasses during the
135 chilling step. To finish, Petri dishes were placed in jars under modified atmosphere (70% O₂,
136 30% CO₂) and stored in a cold room at 6°C, *i.e.* approximatively the average temperature of
137 consumer refrigerators (Roccatto et al., 2017). The practical indicated shelf life of chicken fillets
138 is 14 days. It was then decided to acquire data a little beyond this duration, *i.e.* up to 17 days of
139 storage. During the storage stage, the gas composition of the jars was measured with Oxybaby
140 M+ (WITT-GASETECHNIK GmbH & Co, Witten, Germany). This control was carried out
141 immediately after filling the jars and regularly during the 17 days of storage to check the stability
142 of the gas content.

143 After each step (scalding, chilling), enumeration of culturable *C. jejuni* cells was carried
144 out in order to determine the viability loss resulting from the application of each stressing step
145 and during storage (0, 2, 3, 6, 10 and 17 days). For that purpose, the poultry pieces were
146 individually introduced in a 60-µm porous stomacher bag with 10 mL of physiological water and
147 crushed with a stomacher for one minute. Serial decimal dilutions were carried out, inoculated on
148 Casa medium, a selective medium for *Campylobacter*, (Biomérieux, Dardilly, France) with the
149 Spiral seeder (EasySpiral Interscience, Saint Nom, France). Plates were incubated for 48 hours at
150 42°C under microaerobic conditions (5% O₂ – 10% CO₂). Loss of viability of *C. jejuni* was

151 assessed and expressed in \log_{10} . A total of 54 experiments (3 strains x 3 independent biological
152 replicates x 3 inocula x stressed vs unstressed) were conducted.

153 During the study, the pH value was not measured. We assumed that change during storage, if
154 any, was similar for all strains and inoculum levels.

155

156 2.3 Statistical analysis

157 Log reduction data were fitted using Weibull models with nls tools package of R (version
158 3.5.1) using the RStudio interface (version 1.1.463). The quality of model adjustment was
159 checked by determining Root Mean Square Error (RMSE) and Akaike Information Criterion
160 (AIC). ANOVA ($\alpha = 5\%$) analyses were performed with XLSTAT software (version 16.16.10) to
161 determine significant differences between factors.

162 Predictions of *C. jejuni* level of contamination after six days of storage were estimated by
163 combining probability density obtained after scalding and chilling steps in slaughterhouses
164 previously analysed (Duqué et al., 2018) with Uniform distributions based on raw data collected
165 in this present study. The probabilistic model was straightforward: level of contamination (in
166 \log_{10} CFU/g) after six days was estimated to be equal to the level at slaughterhouse release minus
167 log reduction after retail storage. In the model, variability and uncertainty associated with log
168 reduction were implemented as follows. A Uniform distribution was built using the lowest and
169 highest strain log reduction as bounds ($\text{Min}_{\text{strain-low}}$, $\text{Max}_{\text{strain-high}}$), where the bounds depended on
170 uncertainty estimated using replicates: $\text{Min}_{\text{strain-low}} = \text{Uniform}(\text{Min}_{\text{min}}, \text{Min}_{\text{max}})$, $\text{Max}_{\text{strain-high}} =$
171 $\text{Uniform}(\text{Max}_{\text{min}}, \text{Max}_{\text{max}})$. To propagate strain variability and uncertainty separately into the
172 models, the mc2d package of R was used.

173 **3. Results**

174 *3.1 Effect of heat and cold stress on C. jejuni log reduction*

175 The initial quantification of control pieces showed that the natural contamination of *C.*
176 *jejuni* was not significant: all values were under the detection limit of 0.4 log₁₀ CFU/g (data not
177 shown). From the pre-culture with an initial concentration of 8; 6 and 4 log₁₀ CFU/g, the mean *C.*
178 *jejuni* log reduction due to heat and cold stress were calculated (Table 1). However, whatever the
179 strain and initial concentration, little or no mean log reduction was observed. Indeed, mean log
180 reduction varied only from 0.00 to 0.28 log₁₀ CFU/g (Table 1).

181 *3.2 Effect of strain and inoculum concentration on the log reduction during cold*
182 *storage*

183 Enumeration was performed after different storage points in order to obtain kinetics of log
184 reduction. Overall, kinetics of log reduction appeared to be similar whatever the strains (Fig. 1).
185 To assess the effect of strain and inoculum concentration, ANOVA analysis was performed at
186 each time (data not shown) except day-17 (numerous values below the detection limit). At day-
187 10, only few values were below the detection limit, and values were set to 0 to enable the
188 ANOVA analysis.

189 The ANOVA pointed out that for the same inoculum, there was no significant difference
190 between strains. However, inoculum concentration had a significant impact on the log reduction.
191 Indeed, from the sixth day, the inactivation level (all strains combined) was significantly higher
192 at the lowest concentration of 4 log₁₀ CFU/g (2.23, standard deviation, ±, 0.61 log₁₀ CFU/g),
193 compared to the concentration of 6 and 8 log₁₀ CFU/g (1.64 ± 1.14 and 0.61 ± 0.22 log₁₀ CFU/g
194 respectively).

195 3.3 *Effect of stress on the log reduction during cold storage*

196 Whatever the strain, no significant difference in *C. jejuni* log reduction during storage was
197 observed between unstressed and stressed bacteria at inoculum concentration of 4 and 6 log₁₀
198 CFU/g, meaning no impact of cell history under these conditions. However, a cell history effect
199 was observed at the highest concentration of 8 log₁₀ CFU/g for the strain C09MJLT205
200 (p<0.001), indeed these stressed bacteria appeared to be more resistant at day-10 during storage
201 when previously subjected to heat and cold temperature (Fig 1b). For instance, after ten days, the
202 log reduction reached 3.19 ± 0.23 log₁₀ CFU/g and 1.57 ± 0.66 log₁₀ CFU/g for unstressed and
203 stressed bacteria, respectively.

204

205 3.4 *Data fitting using primary models*

206 For each experimental condition (3 strains x 3 inocula), three biological replicates were carried
207 out, that means in total 27 log reduction curves for stressed conditions, 27 for unstressed ones.
208 The Weibull model was used to fit log reduction kinetics. The Weibull model takes into account
209 the parameter p, corresponding to the shape of the curve (concave for p>1 or convex for p<1) and
210 δ (decimal reduction rate). The equation is described below.

211
$$\log_{10}(N) = \log_{10}(N_0) - \left(\frac{t}{\delta}\right)^p \quad (\text{Eq 1})$$

212 Where log₁₀(N) is the bacterial concentration at time t (days) in CFU/g and log₁₀(N₀) is the initial
213 bacterial concentration in CFU/g.

214

215 First of all, the Weibull model was applied with parameters p and δ estimated for each log
216 reduction curve. In this way, each curve was associated with one value of p and one value of δ.
217 However, for a given microbial population, at the same physiological state, the Weibull model

218 shape parameter p value should be constant (Couvert et al., 2005). In this study, even between
219 biological replicates, this parameter was shown to vary greatly, associated either with concave or
220 convex shapes of the log reduction curves (Supplementary Material Table 1). For instance, for
221 the strain C97anses640 at a concentration of $4 \log_{10}$ CFU/g, p varied between 0.52 and 1.21.
222 Nevertheless, an attempt was done to impose the same p value in the model for each independent
223 replicate (same strain and level of inoculum, same stress condition). The quality of model
224 adjustment was checked by determining Root Mean Square Error (RMSE) and AIC. Both
225 goodness of fit criteria showed clearly that a model adjusted to the data considering p constant for
226 replicated conditions was not suitable to our dataset (Supplementary Material Table 1). It was
227 then decided to estimate the log reduction after storage under chilled conditions using the raw
228 data themselves instead of using estimated values based on Weibull model.

229

230 *3.5 Prediction of C. jejuni level at the end of the chilled storage*

231 It was decided to predict the contamination level of *C. jejuni* at a specific storage point,
232 *i.e.* day-6, because this point was associated with no censored data (no data below the detection
233 limit) and also because it was assumed to be representative of a reasonable time of storage either
234 at retail and/or at home. The contamination level of *C. jejuni* on chicken carcasses after the
235 chilling step, ($N_{\text{post-chilling}}$) was recovered from previous published work (Duqué et al., 2018).
236 Only June to December data (worst-case level of contamination) were kept in the present
237 analysis. Likewise, data collected on chicken legs were used as considered more representative
238 from a French consumer point of view. The mean value of $N_{\text{post-chilling}}$ was estimated to be 2.06
239 \log_{10} CFU/g (Fig. 2), the 2.5th percentile to 0.15 \log_{10} CFU/g and the 97.5th percentile to 3.97
240 \log_{10} CFU/g.

241 In our present study, it was shown that the application of stressing steps could have an
242 impact on the log reduction, depending on the initial concentration of *C. jejuni*. Indeed, log
243 reduction of stressed cells, all strains combined, was shown to get higher for the concentration of
244 $4 \log_{10}$ CFU/g compared to other concentrations. Therefore, to reflect as much as possible the
245 reality of the level of contamination on carcasses, the log reduction data observed on chicken
246 fillets inoculated with the lowest concentration, *i.e.* $4 \log_{10}$ CFU/g, were used for predictions. In
247 other words, it was assumed that the inactivation after 6 days observed with the inoculum of 4
248 \log_{10} CFU/g was an acceptable proxy for predicting what would be the log reduction with an
249 initial contamination varying from 0.15 to $3.97 \log_{10}$ CFU/g. After 6 days of storage, depending
250 on the strain, the mean log reduction varied from 2.00 to $2.68 \log_{10}$ CFU/g (Table 2).

251 Finally, predictions of *C. jejuni* level at the end of the chilled storage was done by
252 combining probability density obtained at slaughterhouses (Duqué et al., 2018) with Uniform
253 distributions set up from raw data collected on chicken in this present study (Table 2). Results are
254 presented in Figure 2. In the variability dimension the contamination varied, after a cold storage
255 under modified atmosphere of six days, from $-2.38 \log_{10}$ CFU/g (2.5th percentile) to $+1.59 \log_{10}$
256 CFU/g (97.5th percentile). The mean contamination level, in \log_{10} , was estimated to be -0.38 with
257 a 95% uncertainty interval of $[-0.92, +0.14]$ (Fig. 2).

258

259 **4. Discussion**

260 The objective of this study was to assess the effect of poultry slaughter process on the log
261 reduction of *C. jejuni* during cold storage in order to predict the contamination level prior to
262 preparation and consumption in the consumer's home.

263 Steps inducing stress from slaughterhouses were identified and then reproduced in laboratory.
264 Food matrix was used to assess the log reduction under chilled storage. This enabled to better

265 reflect the reality as it is known that the log reduction of *C. jejuni* is different between food
266 matrix and lab medium (Membré et al., 2013). In a previous work conducted in our laboratory
267 facilities, the same experiments were carried out using lab medium instead of food product
268 (Duqué et al., 2019). In lab medium, depending of the strain, at a concentration of 8 log₁₀
269 CFU/mL, log reduction after 7 days of chilled storage could reach 3.3 log₁₀ CFU/mL, results
270 similar to those obtained by Garénaux et al. (2008). In contrast, on chicken fillets, in the present
271 study, the log reduction rate did not exceed 1.75 log₁₀ CFU/g for a concentration of 8 log₁₀
272 CFU/g even after 10 days of chilled storage.

273 In laboratory medium, the effect of cell history was highlighted, *i.e.* the influence of heat
274 and cold stress applied successively on the consecutive log reduction during cold storage under
275 modified atmosphere (Duqué et al., 2019). Moreover, log reduction during chilled storage was
276 shown to be strain dependent, whereas on chicken fillets, no strain effect could be observed.
277 When the three strains of *C. jejuni* were exposed to different stresses (heat, cold and oxidative) in
278 a synthetic medium, *C. jejuni* C97anses640 appeared to be less resistant than the two other strains
279 (Duqué et al., 2019). Besides, it was also shown that log reduction during chilled storage was
280 higher if cells had been previously submitted to heat treatment, especially at the highest
281 temperature tested, as opposed to unstressed cells. In contrast, in this study, it appeared that only
282 the strain C09MJLT205 became more resistant during cold storage than the unstressed strain.
283 This difference is related to the cellular history of bacteria. After undergoing stress, such as heat
284 stress, bacteria may adapt and become more resistant in order to survive the next stressing step. It
285 is also important to notice that the difference of response between stressed and unstressed
286 bacterial cells appeared only with the highest inoculum concentration. Increased resistance
287 observed in dense bacterial suspensions might be expected from higher frequency of resistant
288 cells in denser cell suspensions (Fernandez et al., 2012; Hansen and Riemann, 1963). El-Shibiny

289 et al. (2009) hypothesized that the presence of one strain may affect the survival of another in
290 situations where the campylobacters are not actively growing. *Campylobacter* are generally not
291 high competitors with regard to the other bacteria naturally present on the chicken fillet.
292 However, Hilbert et al. (2010) highlighted that this interaction depends on the predominant
293 microbiota after two weeks of storage. Indeed, after this time, the interaction was advantageous
294 for *C. jejuni*. Besides, El-Shibiny et al. (2009) also indicated that the detrimental presence of
295 competing microbiota to *Campylobacter* may be strain dependent. These findings highlighted the
296 importance of considering strain variability in exposure assessment and to conduct tests in food
297 rather than in broth. Altogether, our results reinforce the fact that bacterial behaviour observed at
298 the laboratory scale cannot always be extrapolated to real conditions, *i.e.* low contamination level
299 in food matrix with various strains. They point out that there may be non-negligible consequences
300 on food safety and risk assessment.

301 Beyond differences between food matrix and lab medium, results were also shown to differ
302 from other studies using poultry meat. Indeed, log reduction induced by thermal stress was lower
303 in this study compared to previous work on thermal inactivation (Berrang and Dickens, 2000;
304 Blankenship and Craven, 1982; Duffy et al., 2014; Koidis and Doyle, 1983; Osiriphun et al.,
305 2012). This difference could be explained by the experimental setup. Indeed, chicken fillets were
306 either put in resealable bag or in a petri dish depending on the stress applied, which can enhance
307 protection regarding desiccation. Bacteria were neither in direct contact with water during heat
308 stress nor to air during cold stress. Indeed, during the scalding step, water could play a role in the
309 detachment of cells. In addition, chilling step can induce stress linked to water activity and air
310 flow. During this study, even if fillets were exposed to air chilling, the ventilation was quite low
311 compared to chilling room from slaughterhouses while it is known that desiccation plays a role
312 on bacterial inactivation. For instance, some studies aimed to assess the log reduction of *Listeria*

313 *monocytogenes* and *C. jejuni* during the chilling step using a miniaturized chilling room
314 prototype. These studies highlighted the impact of the relative humidity and the air flow on log
315 reduction (Rivoal et al., 2005; Zoz et al., 2016).

316 One attempt to use predictive microbiology to predict the contamination level of *C. jejuni* at
317 the consumer's home was performed. However, we faced a high variability associated with the
318 Weibull p parameter even between biological replicates while p is generally reported to be
319 constant when the same conditions are applied (Couvert et al., 2005). The parameter p can be
320 considered constant for a given strain in a given physiological state if the environmental
321 conditions are constant. In our study, the chicken fillet cannot be considered having perfectly
322 identical environmental conditions as they are biological materials. A small change of
323 composition could affect the meat buffer effect and then slightly the pH value of the meat for
324 instance. Besides, although much lower in concentration than the inoculated cells, the natural
325 *Campylobacter* strains present on chicken are likely better adapted to chicken and more securely
326 attached to the meat, they bring additional heterogeneity, and could also explain the difference
327 between biological replicates. In absence of satisfactory predictive model to describe log
328 reduction kinetics, we decided to use directly biological raw data without fitting models. Of
329 course, that has some limitations: rather than using the whole kinetics, estimation of the log
330 reduction was performed for a specific time storage point. Despite this lack of flexibility, we
331 were able to estimate the contamination level of *C. jejuni* after a realistic storage time. Moreover,
332 we included in our analysis, uncertainty and variability separately as recommended in risk
333 assessment (Nauta, 2000; Thompson, 2002). In MRA, it is generally admitted that uncertainty
334 comes from both biological data and predictive model adjustment (Haas et al., 2014). Uncertainty
335 was estimated from biological replicates considering that with more replicates, uncertainty will
336 be reduced, variability came from the three strains studied here knowing that these strains are

337 representative of the strain diversity found on chicken (Colles et al., 2003; De Haan et al., 2010;
338 Dingle et al., 2002; Duqué et al., 2019; Guyard-Nicodeme et al., 2015; Habib et al., 2009;
339 Levesque et al., 2008; Ragimbeau et al., 2008; Sheppard et al., 2009).

340 This quantification enabled to determine if a Performance Objective (PO) regarding *C. jejuni*
341 could be met. An FSO has been suggested for the consumption of chicken meat contaminated
342 with *C. jejuni*. The FSO translates the public health risk into a definable objective: a frequency or
343 maximum concentration of a microbiological hazard in a food at the time of consumption.
344 Zwietering (2005) has described a scheme for the calculation of a food safety objective (FSO)
345 from an ALOP (Appropriate Level of Protection). Thus, an FSO of $-4.15 \log_{10}$ CFU/g, a
346 concentration of *Campylobacter* that should not be exceeded of cooked chicken meat at the
347 consumption time has been determined (ICMSF, 2018). From the FSO, assuming a negligible
348 cross contamination at home, a PO for the level of *Campylobacter* on raw chicken before it enters
349 the consumer's home has been calculated. This value would be $2.55 \log_{10}$ CFU/g considering a
350 cooking step delivering at least a $7 \log_{10}$ reduction (ICMSF, 2018), this latter value was
351 recommended by the U.S FDA after having consulted the experts of the US National Advisory
352 Committee on Microbiological Criteria for Foods (NACMCF) regarding *Salmonella* log
353 reduction. Since *Campylobacter* is more heat susceptible, recommendation for *Salmonella* would
354 also result in 7 log-reduction for *Campylobacter*.

355 Thus, the contamination level estimated after 6 days of storage had to be lower than the
356 assumed PO. From our estimates, chicken legs stored for 6 days at 6°C (range between -2.38
357 \log_{10} CFU/g to $+1.59 \log_{10}$ CFU/g) comply with the assumed PO of $2.55 \log_{10}$ CFU/g and
358 consequently, chicken legs stored for 6 days at 6°C and then cooked to be immediately
359 consumed, should not exceed the FSO. This conclusion is based upon estimation and even if care
360 has been taken to include uncertainty and variability in the calculations, it cannot be considered

361 as definitive. As mentioned by the ICMSF: "*the ALOP, FSOs and POs calculated in the*
362 *following sections should not be applied in practice without further consideration*". Moreover,
363 we should keep in mind that conditions used in this study (temperatures used, stress conditions)
364 were inspired from French slaughterhouses. Hence, all results could only be applied and used in
365 France. Besides, experiments were carried out on leg meat. The pH and fat composition between
366 leg and breast meat differs significantly and this may limit furthermore the generalisation.
367 In conclusion, to better predict *Campylobacter* contamination in poultry processing, it is essential
368 to take into account all sources of variability that may influence inactivation, such as initial
369 concentration, processing steps or strains, and to separate variability from uncertainty due to lack
370 of data, poor fitting models or both. With the recent expansion of omic methods, it might be
371 possible to decipher better this variability, without generating additional uncertainty, and
372 continue improving the predictions of pathogen levels at factory release, retail or consumer place.
373 That is definitively valuable to obtain more accurate risk assessment predictions and move
374 towards risk-based food safety management.

375

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380

381 **Conflicts of interest**

382 The authors declare that the research was conducted in the absence of any commercial or
383 financial relationships that could be construed as a potential conflict of interest

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525 **Table 1** : Mean log reduction of three strains of *C. jejuni* expressed in $\Delta\log$ (\log_{10} CFU/g) and
 526 standard deviation associated after application of heat and cold stress.

Inoculum concentration (log CFU/g)	C97anses640		C09MJLT205		RM1221	
	heat stress	cold stress	heat stress	cold stress	heat stress	cold stress
4.00	0.21 ± 0.09	0.09 ± 0.17	0.00 ± 0.15	0.03 ± 0.06	0.00 ± 0.09	0.01 ± 0.12
6.00	0.26 ± 0.14	0.13 ± 0.04	0.28 ± 0.19	0.03 ± 0.07	0.14 ± 0.14	0.04 ± 0.05
8.00	0.27 ± 0.02	0.00 ± 0.05	0.08 ± 0.08	0.10 ± 0.40	0.05 ± 0.07	0.03 ± 0.05

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528

529 **Table 2:** Log reduction of three strains of *C. jejuni* expressed in $\Delta\log$ (\log_{10} CFU/g) and standard
 530 deviation associated after application of heat and cold stress followed by six days of storage at
 531 chilled conditions. Results extracted from data collected at low inoculum ($4 \log_{10}$ CFU/g).

Concentration (log CFU/g)	C97anses640	C09MJLT205	RM1221
	2.35	1.96	2.79
Three replicates	3.38	1.62	1.83
	2.31	2.45	1.38
Mean	2.68	2.01	2.00
SD	0.60	0.42	0.72

532

533 **Figures legends**

534 **Figure 1.** Inactivation kinetics of C97anses640 (a), C09MJLT205 (b) and RM1221 (c) *C. jejuni*
535 strains according to three inoculum concentrations (4, 6 and 8 log₁₀ CFU/g). Error bars represent
536 standard deviation associated with each point. In solid lines are represented kinetics when strains
537 subjected to stresses and the control (unstressed) in dotted lines. Horizontal line represents the
538 limit of detection due to enumeration method and open symbols represent values below the
539 detection limit. * (p<0.001) when comparing stressed and unstressed cells.

540

541 **Figure 2.** Predicted contamination level of *Campylobacter jejuni* on chicken carcasses based on
542 samples collected on leg skin for the period from June to December (Curves on the Right) and
543 contamination level of *Campylobacter* on chicken filets after six days (Curves on the Left) with
544 their uncertainty interval. After six days, the contamination level varied from -2.4 to 1.6 log
545 CFU/g (2.5 and 97.5 percentile mean values, respectively) in the variability dimension. The mean
546 value of the contamination level after six days was estimated at -0.4 log CFU/g (red line). This
547 mean concentration was estimated with a 95% uncertainty interval of [-0.9, +0.1] (dotted lines).

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Figure 1

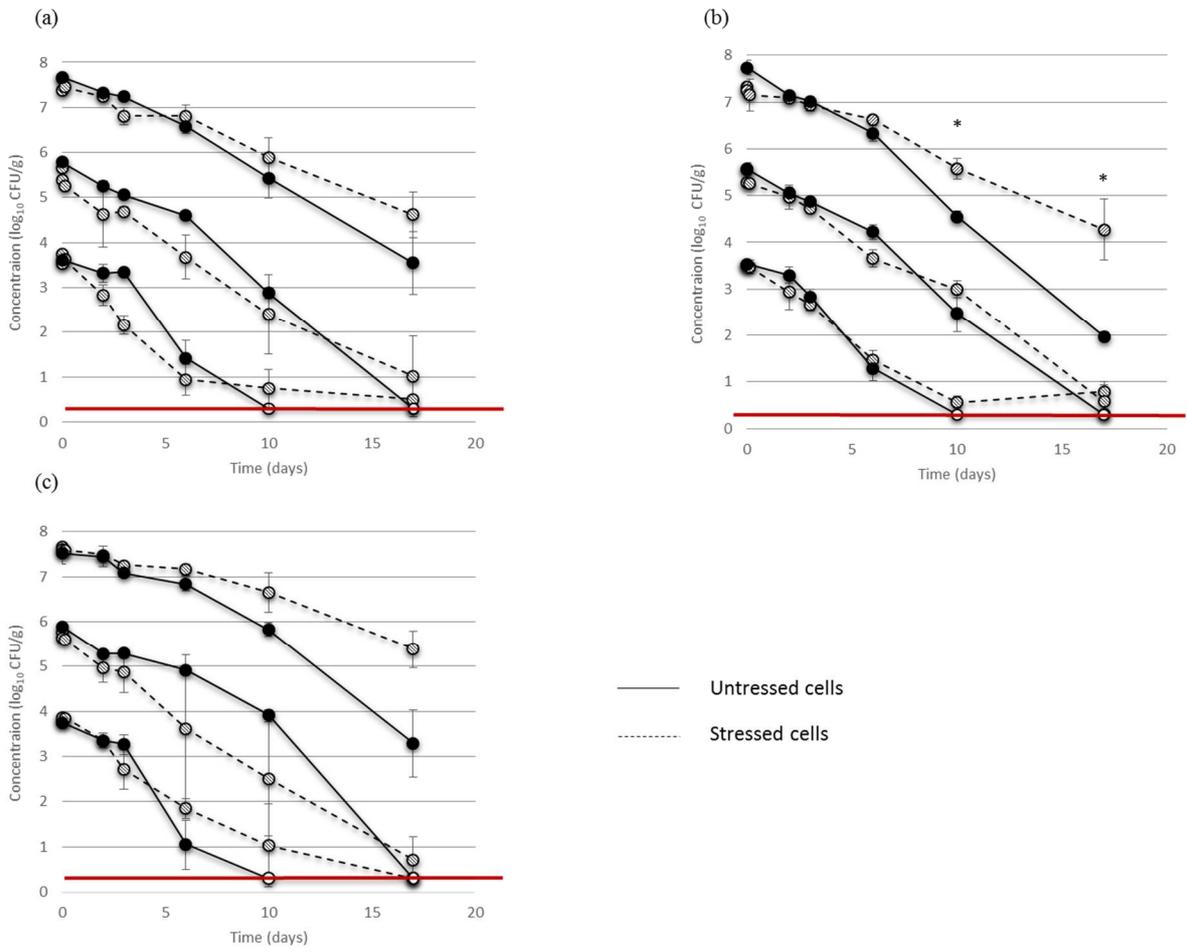


Figure 2.

