FaBE 2013 International Conferences on Food and Biosystems Engineering

(SET) (CD-ROM) ISBN: 978-960-9510-09-7

Proceedings of FaBE 2013 International Conferences on Food and Biosystems Engineering

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Skiathos, 30 May - 02 June 2013, GREECE.

[Volume 2]

[vol. 2] ISBN: 978-960-9510-11-0

Technological Educational Institute of Thessaly

Greece. **[vol. 2] ISBN: 978-960-9510-11-0**

[eng]

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Published by the **Technological Educational Institute of Thessaly (TEI of Thessaly), [Greece].**

All papers of the present volume were peer reviewed by two independent reviewers. Acceptance was granted when both reviewers' recommendations were positive.

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Food and Biosystems Engineering

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A META-ANALYSIS OF THE BETWEEN-BATCH VARIABILITY IN THE EFFECT OF CHILLING ON THE *SALMONELLA* INCIDENCE ON PIG CARCASSES

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ABSTRACT

The objective of this work was to study the effect of chilling on the occurrence of *Salmonella* on pig carcasses at batch level by meta-analysis. Fixed-effects and random-effects meta-analysis were conducted, and the random-effects solution was preferred to account for the significant variability in effect size estimated from 51 sampled batches extracted from 13 primary studies. This study results indicated that chilling reduces the *Salmonella* incidence on pig carcasses by a mean ratio of ~1.92 (95% CI: 1.36 – 2.70). Multilevel meta-analyses models investigating study characteristics that could explain the heterogeneity (τ^2) in the true effect size among sampled batches (τ^2=0.373), revealed that 'total sample size' and 'carcass swabbed area' impact ($p<0.05$) on the measured effect size of chilling. The fact that swabbed area explained 62% and total sample size 38% of the total heterogeneity in the chilling true effect size, gives rise to an awareness that differences in experimental design greatly affects our substantive conclusion about the effect of chilling on *Salmonella* recovery. Higher swabbed areas and greater sample sizes led to more precise and greater estimates of the decreasing effect of chilling on *Salmonella*.

Keywords: Pig, slaughterhouse, meta-analysis, chilling, *Salmonella*.

1. INTRODUCTION

Meta-analysis concerns the statistical summarisation of the results of a large collection of independently conducted primary studies on one specific research question. In a fixed-effects approach, combining studies is simple as they can be regarded as direct replications of each other and one can assume that the possible differences between study outcomes are due to

sampling error. However, heterogeneity in primary study outcomes is expected as different studies employ different sampling methods and different experimental manipulations. To address this heterogeneity, a random-effects model is the best choice as it assumes that study outcomes vary not only because of random sampling effects, but also because of real differences between studies. If heterogeneity among primary studies is present, the next goal of meta-analysis is to attempt to identify the study characteristics or moderators that explain the differences between study outcomes. These moderating variables can be research design features, data collection procedures or type of subjects sampled, and can be assessed using a *multilevel analysis*, with subjects between studies at the first level and studies at the second level.

In food safety, the results of a potential intervention strategy might be conflicting among primary studies, and different studies normally fail to provide the same level of confidence for effectiveness because of differences in study design, statistical power or sample size. Thus, meta-analysis becomes useful in the field of food safety for the identification, appraisal and summarisation of results from large quantities of research. The objectives of this research were: (i) to compile all the published findings on the effect of chilling on *Salmonella* occurrence on pig carcasses, and quantitatively summarise these outcomes at batch level; (ii) and, if heterogeneity is present among studies, to evaluate its causes by means of a multilevel meta-analysis using coded study characteristics such as total sample size and swabbed area.

2. METHODOLOGY

Electronic searches were carried out to identify published primary studies. After assessing all the information presented in every study, eight primary studies were considered appropriate for inclusion as they presented the quantitative data broken down by batch. (i.e., sampling visit). Primary studies that presented only pooled results were not considered. A total of 51 batches were then extracted from the eight primary studies. After data collection, a parameterisation of the intervention's effect size needs to be determined. The effect size (θ) refers to the degree to which the hypothetical phenomenon (i.e., decrease in *Salmonella* prevalence due to chilling) is present in the population (i.e., pig carcasses during processing at slaughterhouses). The parameter measuring the effect size of an intervention is a common metric that permits direct comparison and summation of primary studies. Because the data

generated by occurrence studies is binary (i.e., a pig carcass tests either positive or negative for *Salmonella*), the chosen parameter to measure effect size was relative risk *(θ=RR*).

2.1. Description of data sets

The outcome data of the 51 batches were available on n_T pig carcasses in the post-chill group (treated group) and n_C pig carcasses in the pre-chill group (control group). The number of successes (*Salmonella*-positive carcasses) in the post-chill and pre-chill group is represented by s_T and s_C, respectively. Table 1 compiles the occurrence data for the batch-level meta-analysis. The study characteristic of 'carcass swabbed area' (*A*) and 'total sample size' ($N=n_C+n_T$) were included in the data set as moderating variables. Three meta-analyses were then conducted on this data: fixed-effects, random-effects and multilevel models with the moderating variables *A* and *N*.

2.2. Fixed-effects meta-analysis

A fixed-effects meta-analysis can be conducted when there is an assumption that the possible differences between study results are due to sampling variance,

$$\theta_j = \Theta + \varepsilon_j \qquad (1)$$

with θ_j the observed effect size in the primary study *j*, Θ the population effect size, and ε_j the residual error due to sampling variance. It is assumed that the ε_j have a normal distribution with mean zero and a true variance ξ^2. So, it follows that for a fixed-effects meta-analysis model, $\theta_i \sim Normal\left(\Theta,\xi^2\right)$.

2.3. Random-effects meta-analysis

Most meta-analyses are based on sets of studies that are not exactly identical in their methods and the characteristics of their samples, which may introduce variability (i.e., heterogeneity) among the true effects. One way to model the heterogeneity is to treat it as purely random, assuming that each study investigates its own true effect size Θ_j,

$$\theta_j = \Theta_j + \varepsilon_j = \overline{\Theta} + v_j + \varepsilon_j \qquad (2)$$

with $\overline{\Theta}$ being the mean true effect size and v_j the deviation of the true study effect size Θ_j from the mean true effect size. The values of v_j are normally distributed random effects with a mean of zero and a variance of τ^2. It follows that for a random-effects meta-analysis model, $\theta_j \sim Normal\left(\Theta,\tau^2+\xi^2\right)$. In this approach, two sources of variation are distinguished:

Table 1. Batch-level occurrence of *Salmonella*-positive pig carcasses before and after chilling as detected in primary studies, with the extracted study characteristic of carcass swabbed area

Coded study	Coded batch	Area of swab (cm^2) (A)	Pre-chill group (Control)		Post-chill group (Treated)		Reference
			s_C	n_C	s_T	n_T	
1	1	1000	13	25	9	25	Booteldoorn et al. (2003)
	2	1000	1	30	0	30	
	3	1000	2	30	3	20	
2	4	300	2	30	1	30	Bouvet el al. (2003)
	5	300	4	30	2	30	
	6	300	0	29	1	29	
	7	300	1	33	2	33	
	8	300	1	30	0	30	
	9	300	0	30	0	30	
3	10	100	0	23	4	23	Cutter (2003)
	11	100	0	30	2	30	
	12	100	2	45	2	45	
	13	100	0	30	0	40	
	14	100	1	30	0	15	
	15	100	1	15	0	15	
	16	100	0	15	0	15	
4	17	1000	7	25	3	25	Davies et al. (1999)
5	18	400	0	21	0	16	Duggan et al. (2010)
	19	400	0	13	0	13	
	20	400	0	16	1	16	
	21	400	1	16	0	16	
	22	400	7	19	0	16	
	23	400	0	16	0	16	
	24	400	5	15	2	15	
	25	400	2	15	1	15	
	26	400	1	14	0	8	
	27	400	1	10	1	10	
	28	400	1	10	0	10	
	29	400	0	10	0	10	
6	30	100	0	30	0	30	Minvielle (personal communication)
	31	100	4	30	0	30	
	32	100	1	30	4	30	
	33	100	7	30	0	30	
	34	100	2	30	3	30	
	35	100	2	30	13	30	
7	36	600	0	20	0	20	De Busser et al. (2011)
	37	600	0	19	0	19	
	38	600	1	22	0	22	
	39	600	1	21	0	21	
	40	600	22	28	4	28	
	41	600	1	23	0	23	
	42	600	4	21	0	21	
	43	600	1	23	1	23	
	44	600	1	24	0	24	
	45	600	0	25	0	25	
8	46	1350	27	100	12	100	Arguello et al. (2011)
	47	1350	57	99	14	98	
	48	1350	34	112	5	112	
	49	1350	9	45	0	44	
	50	1350	36	50	11	49	
	51	1350	16	40	6	44	

sampling variation (ξ^2) and variation between true effect sizes (τ^2). By including this additional component (τ^2), the standard error in the effect size estimates represents random variability at both the subject level and the study level.

2.4. Multilevel meta-analysis

If the between-study variance τ^2 is shown to be noteworthy, study characteristics or moderators can be added to the model to account for at least part of the heterogeneity in the true effects. This leads to the mixed-effects model given by,

$$\theta_j = \Theta_j + \varepsilon_j = \beta_0 + \sum_{s=1}^{S} \beta_s X_{sj} + v_j + \varepsilon_j \qquad (3)$$

with X_1 to X_S S study characteristics. This model treats the moderator effects β_s as fixed and the v_j as random effects that distribute normally with a mean zero and a variance of τ^2. Yet, τ^2 now denotes the amount of residual heterogeneity among the true effects, or the variability among the true effects that is not accounted for by the S moderators included in the model. The goal of the analysis is then to examine to what extent the moderators influence the size of the average true effect size Θ. Meta-analysis models were fitted in R version 2.14.2 (R Development Core Team) using the 'metafor' package (Viechtbauer, 2010), which provides functions for fitting the three models described above.

3. RESULTS AND DISCUSSION

The forest plot shown in Figure 1 highlights the variability in effect size estimates and precision among studies; and the marker size illustrates the contribution of each study (weight) to the overall effect estimate. A visual examination of the forest plot gives an idea of the discrepancy among outcomes, with 10 out of 51 batches reporting increase in *Salmonella* occurrence during chilling. This is not surprising given the several sources of variability among studies and abattoirs such as sampling site, extent of swab, chilling equipment, cross contamination of carcasses, level of *Salmonella* infection at slaughterhouses, differences in the microbiological protocol, season, year and country, among others. The heterogeneity in the measured log relative risks between batches (τ^2=0.578; Table 2) was statistically significant, as attested by the Qtest (Table 2), and hence it should be accounted for. Thus, the random-effects meta-analysis was of significantly better fit than the fixed-effects model, as indicated by the lower Bayesian Information Criterion (BIC). This model suggests that

chilling reduces the occurrence of *Salmonella* in pig carcasses by a factor of ~1.92 (95% CI: 1.36 – 2.70) (taking the inverse of the exponential of the estimated overall effect size Θ = -0.648).

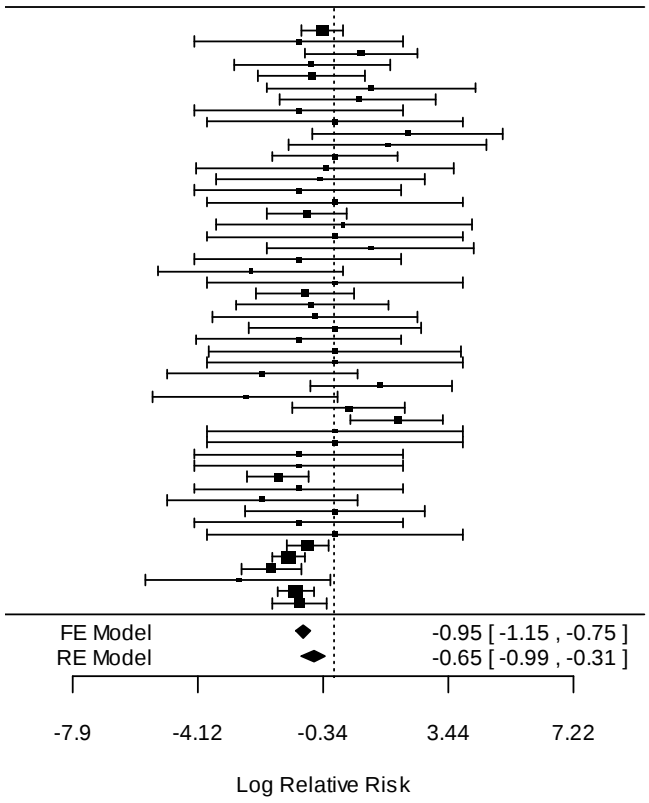

Figure 1. Forest plot of the risk of *Salmonella* occurrence on pig carcasses after chilling relative to before chilling among surveyed production batches. Individual estimates and overall fixed and random effects are shown with 95% confidence intervals

The power of multilevel meta-analysis becomes apparent when attempting to explain the heterogeneity in the study outcomes. It was hypothesised that with larger swab areas (A) and with larger studies in terms of total sample size (N), the observed effect size of chilling on *Salmonella* occurrence would become more precise; and that at least part of the heterogeneity found between studies could be explained by the differences in those experimental design moderating variables. The estimates of the effect size of chilling depend significantly on the extent of the carcass swabs, as indicated by the significant coefficient β_1 and the significant QM test for the moderating variable (Table 2). The value of the β_1 indicates that an increase in one cm^2 in swabbed area corresponds to a reduction of -0.001 units in terms of the average log relative risk. The estimated amount of residual heterogeneity is $\tau^2 = 0.145$, suggesting that

61% (0.373-0.145)/0.373) of the total amount of heterogeneity between studies could be accounted for by including the 'swabbed area' in the model. The total sample size, although a significant moderating variable, had less predictability than the swabbed area, as its inclusion in the multilevel model explained just about half of the between-study heterogeneity ((0.373-0.230)/0.373 = 38%) that the swabbed area moderator explained.

Table 2. Results of the batch-level meta-analysis models for the natural logarithm of relative risk of *Salmonella* presence on pig carcasses after chilling in relation to before chilling

Model	Fixed-effects	Random-effects	With moderator:	
			Swabbed area	Total carcasses
# Entries (J)	51	51	51	51
Parameters				
Intercept	-0.948 (0.103)***	-0.648 (0.172)***	0.111 (0.283)^{ns}	-0.181 (0.267)^{ns}
Swabbed area			-0.001 (0.0003)***	
Total carcasses				-0.006 (0.0025)*
Heterogeneity				
Q test	64.16 (df=50)·			
τ^2		0.373	0.145	0.230
QM moderators			11.92 (df=1)***	5.54 (df=1)*
QE residual heterogeneity			46.34 (df=49)^{ns}	52.65 (df=49)^{ns}
Goodness-of-fit				
BIC	179.11	176.57	184.63	185.59

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '·' 0.1 '^{ns}' Non-significant

To facilitate the interpretation of the moderators, predicted average log relative risks as a function of the swabbed area and the total sample size are shown (Figure 2). The observed log RR values are drawn proportional to the weights, and predictions are shown with corresponding 95% confidence interval bounds. These plots illustrate how as swabbed area and total sample size increases in the experimental design of a primary study, the observed effect size tends to be lower in terms of log relative risk (i.e., higher observed reduction ratio of *Salmonella* occurrence due to chilling). On the other hand, when both swabbed area and total sample size were small, the effect size values from the primary studies were more scattered and even conflicting, meaning that the measures were highly imprecise. Notice that when a carcass area of 100 cm^2 was swabbed, the chilling effect size in log relative risk as estimated from the different surveys, varied from -2.8 to 2.2. Likewise, when the total sample size taken in a batch was equal or lower than 60 pig carcasses, the chilling effect sizes measured by the studies were very disperse ranging between -2.8 and 2.2. Thus, it is not coincidence that as the swabbed area and total sample size increased, the weights assigned to

the outcomes became larger (Figure 2). The highly imprecise results when sample size and swabbed area are low may be explained by the fact that, although *Salmonella* viability has been proven, at least at laboratory level, to be affected by both temperature (cold shock and refrigerated storage) and water activity (osmotic shock); still the efficacy of the chilling

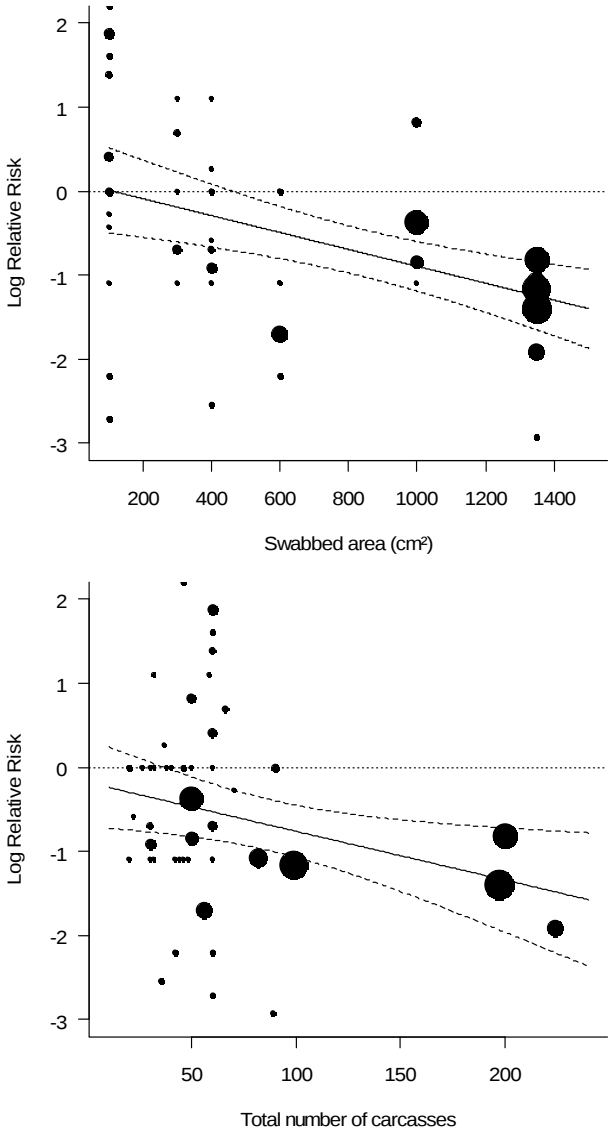

Figure 2. Effect of the carcass swabbed area (top) and the total sample size (bottom) on the estimation of the risk of *Salmonella* occurrence on pig carcasses after chilling relative to before chilling, as modelled by separate multilevel meta-analyses

operation for the reduction of *Salmonella* is also affected by other equally important factors, related to the chilling systems, abattoir logistics, cross-contamination, abattoir hygiene, etc. In addition, *Salmonella* cells are not homogeneously distributed on carcasses, which will greatly

add to the *uncertainty* in the measured outcomes (this is, although a pre-chill carcass may contain *Salmonella* cells, swabbing a *Salmonella*-free area will lead to a negative result). On the other hand, the fact that pre-chill and post-chill measurements were mostly performed on different carcasses adds extra randomness to the measured outcome. Thus, it is then expected that, with so many factors affecting the performance (and the measurement itself of the performance) of the chilling operation, the study size will have a strong influence on the measured effect size. From the multilevel meta-analyses, it can also be deduced that, if we were to conduct a survey study in one abattoir only, a well-designed experiment that has the *minimum* statistical power or resolution to produce consistent and reliable results evidencing the chilling effect, would consist of sampling a total of 60 pig carcasses (30 pre-chill carcasses and 30 post-chill) per batch, with swabs of at least 500 cm^2.

4. CONCLUSION

A random-effects meta-analysis conducted on the results from 51 sampled batches demonstrated that chilling reduces significantly the *Salmonella* incidence on pig carcasses by a mean factor of ~1.92 (95% CI: 1.36 – 2.70). However, study characteristics such as the 'total sample size' and 'carcass swabbed area' significantly affect the measured effect size of chilling extracted from the primary studies. This finding represents a warning that the total sample size and swabbed area, as defined in an experimental design, pose major threats to our substantive conclusion about the effect of chilling on *Salmonella* incidence. Small-size studies (sample size lower than 60) and small swabbed areas (lower than 500 cm^2) may lead to imprecise and even conflicting conclusions of increase or decrease of *Salmonella* incidence due to chilling. Thus, multilevel meta-analysis was also instrumental in the definition of what should be a well-designed study that has the *minimum* statistical power to produce precise results of the decreasing effect of chilling on *Salmonella* occurrence on pig carcasses.

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